

Keeping narcotic drugs at bay

The United States has embarked on what seems to be a competition to find the toughest recipe for keeping hard drugs out of reach. But the remedies likely to be most effective may well be overlooked.

NARCOTIC drugs seem destined to be an issue in this November's mid-term congressional elections in the United States, but nobody is quite sure why. The death in August from cocaine intoxication of a talented college basketball player must have had something to do with this development. So too has the spate of recent publicity for a form of cocaine called "crack" (the active principle without its molecular attachments), said to be more rapidly effective, more addictive and cheaper than the old-fashioned coke of the 1920s, also recently back in fashion. President Reagan (whose wife has done a valuable job in persuading people that there is a problem to be tackled) has now decreed that "sensitive" federal employees should be tested for the use of drugs (for some of the practical difficulties, see page 285) and, in his folksy way, has provided a urine sample for analysis. (So, too, has vice-president George Bush, while the refusal of the mayor of Atlanta to do so on the grounds of civil liberties may have been a contributory factor to his failure earlier this month to win nomination as a candidate for the US Senate.) Where will it all lead?

There is a danger that Gresham's law, the doctrine that bad money displaces good, will apply. Legislators of all kinds are busy with bills at various stages, with the House of Representatives way out in front. On 11 September, its members (each of whom is up for election in November) endorsed a rag-bag of measures intended to keep narcotics out of the United States which would have the administration do more of what it is doing already, but would also introduce mandatory death sentences for those repeatedly convicted of selling drugs and would require that the US military forces should be used to police the frontiers of the United States with such thoroughness that the illicit import of drugs would be halted in 45 days. The US Senate lags in the race, but seems to have fallen in with the notion that there should be an agreed piece of legislation by November. The administration's own proposals, financially more modest but linked with the scheme for testing federal employees for drug usage, are permissively agnostic on the wilder additions to the sensible parts of the House of Representatives' bill. One way or another, some amalgam of these proposals is almost certain to become law.

Teenagers

That there is a haunting problem to be tackled is beyond dispute. Although the number of heroin users in the United States appears to have reached a plateau in the past year or so, there are still some 500,000 people in this condition known to the several health agencies concerned with their treatment and rehabilitation (such as it is), or more than twenty times as many as there are AIDS patients. Cocaine seems to be less widely used, but can evidently be addictive and fatal. Crack is especially feared because it has been used among groups of teenagers, although the extent to which the pushers have penetrated this vulnerable part of their market remains unknown. Other industrial nations, or at least the nations of Western Europe, had better take note of the way the wind is blowing in the United States.

What might more moderately be done? One of the surprising

features of the past two months of excitement about narcotics in the United States is the degree to which it is uninformed by data. Nobody is much to blame for that, for illicit activities are almost always also secret. Yet it should be possible to learn more than is known at present about the pattern of drug use in the United States and elsewhere. If, for example, it is true that the recruitment of new heroin addicts has been declining, however great the number still using the drug, it would be valuable to know why. Have people switched to other things, cocaine perhaps? Or have they been influenced by the public education of the past several years? Or are some potential users becoming sensible spontaneously, and if so why? These are the kinds of questions that might have been tackled by the type of social science that became unfashionable in the late 1970s. To the extent that all the proposals now in the legislative mill in the United States advocate that more should be spent on "research", it must be hoped that some of the money will be directed in these constructive directions.

Crisis

The general nature of the outcome of inquiries such as these is, uncomfortably for those up for re-election, predictably unclear. There can be no simple remedy for the drugs crisis in the United States, and for its lesser images elsewhere, unless it is that the word "crisis" should be prohibited in this connection. If, by magic, the US forces were to do what congressmen wish, and halt the flow of cocaine from Latin America, would not those who must get high go back to marijuana, in the production of which the United States is said now to be half self-sufficient? If some drug-sellers were found, after the inevitable legal delays, to qualify for the death penalty under the rules now being talked of, is there any reason to expect that such a move would deter people now from taking to this unwholesome trade, often under the compulsion of their own addiction? The sad truth is that there can be no quick fix and that the best hope is persistence.

Two particular issues remain. While the US Congress and the administration seem to have set aside the Gramm-Rudman injunction to reduce the budget deficit when planning their fight against drugs, centres for the treatment and rehabilitation of drug-users are too few and inadequately provided; while necessarily the responsibility of state governments, federal support for them could work wonders but comparatively little is now promised. Similarly, the drug-testing programmes promised (some say threatened) by the federal government, themselves formal versions of programmes operated by several large corporations, are specified in strictly negative terms (which is why they are denounced as illibertarian). What is a person's right of appeal against a false positive, for example? And given that the drugs problem is in the broadest sense a social problem, one in which narcotics users may fairly claim to be partly the victims of past neglect, what will be done to help them as well as to punish them (by the sack)? It is not entirely irrelevant that these days the chief overseas sources of the supply of narcotics to the United States are mainly poor farmers in countries that might have expected more past aid programmes than, in the event, they received. □



Trade makes sense

Against the odds, last week's meeting of the trading nations reached a hopeful agreement.

THE General Agreement on Tariffs and Trade (GATT) is not so much a treaty whose violations lead nations to declare war on each other as a kind of bargain which is repeatedly broken by nations large and small, but which miraculously has not yet been so flagrantly violated as to be torn up. On the contrary, GATT and its consequences have helped us all survive the long recession of the past decade. While economic growth has been stagnant in most places and almost stagnant even in Japan, the growth of world trade has been spectacular, roughly 7 per cent a year.

This steady trend has been more than merely an exercise in accountancy in which some countries have been able to pay for imports they could not otherwise have afforded. Rather, it is a sign that, even on an international scale, there is still substance in Adam Smith's great dream of an efficient (or economic) "division of labour" in which people concentrate on doing what they do best, selling the product of their labour to others and spending the proceeds on the products of other specialists' labours. While the prosperity of nineteenth century Britain was a mark of how the principle could successfully augment the wealth of a single nation, it is far too soon to guess how the prosperity of all nations will be increased by a full-scale extension of the principle internationally. In fact that process has hardly begun.

That may be why it was generally expected that the meeting last week in Uruguay of GATT's seventy-nine members would end in failure: the notion that free trade creates economic resources is widely disbelieved. But there was a surprise. In the event, the meeting accomplished most of what was expected of it. There are to be new negotiations on the tariffs by which some countries seek to protect domestic industries. (GATT's rules at present do not outlaw tariffs, but do require that tariffs should not discriminate between competing suppliers and that, eventually, they should melt away.) More important, there are to be negotiations on agriculture (which offers Europe's best hope of shedding its cumbersome Common Agricultural Policy), on trade in services (such as insurance policies) and on the protection of intellectual property.

The credit for this agreement rests with the United States, which has freely used the threat that the US Congress would thrust a slew of protectionist legislation on the administration if the meeting in Uruguay flopped. But the inevitable protests at "dollar imperialism" that will now be made should be scorned: US salesmen will indeed benefit if the planned negotiations succeed, but so too will the salesmen of other countries. Adam Smith, if he were still alive, would say that trade is not a zero-sum game.

None of this implies that the time has come to celebrate; years of negotiations about details lie ahead. Some of the issues yet to be decided have vital importance for the technical community, not least the issue of what is grandly called intellectual property, which meant copyrights in printed books in Adam Smith's time and now means patents and designs as well. The United States was entirely right last week to argue that the international extension of the doctrine of the division of labour requires a similar extension of the principle that innovations (and inventors) deserve protection from pirates.

The snag is that even nationally, this right is not absolute; many industrialized countries reserve the right to grant compulsory licenses for drugs they consider to be socially important, while a wide group of governments (the beneficiaries of the Paris amendment of the Berne Copyright Convention) has the right to assign others' copyrights if they consider that justified in the interests of education or research. As in other arguments, each side has a case. Just where to draw the line is certain to perplex

GATT in the years ahead.

The predictable quarrels about intellectual property will be suffused in part with the other perennial problem in GATT: the economic imbalance between the industrialized and the developing world. In principle, the economic division of labour should make each more prosperous, but there is nothing in Adam Smith to legislate for the abolition of even relative poverty. But the developing world justly complains that even under the present version of GATT, industrial members are far too active in the protection of their own industries against imports from poor countries. The notorious Multi-Fibre Agreement (an agreed exception to GATT rules) keeps rich workers employed in textile factories when they might be making computers instead, at the expense of their compatriot consumers and of would-be textile workers abroad. Is it any wonder that the latter should turn to piracy? And is there a chance that, in the negotiations that lie ahead, the industrialized nations will see the logic of the trade-off they must make between the legitimate demand for protection for their innovations and the improper protection they at present secure for textiles, shipbuilding, steel and other commodities at the cost of impoverishing developing countries that could make them more cheaply? □

Fireworks at Yale

Yale University, in the best traditions, seems to have found itself a firebrand for a president.

THE inauguration at the weekend of Benno C. Schmidt as the new president of Yale was predictably a lively occasion. At 42, Schmidt is young as these appointments go, and while his attainments at Columbia as a constitutional lawyer are as distinguished as his academic colleagues would expect, he also has something of a reputation as an accomplished performer on television. His inaugural address was an extended version of a brief intervention at the Harvard ceremonies two weeks earlier: universities' social function includes irreverence, which institutions elsewhere (but especially governments) detest and are forever trying to suborn. Schmidt deplored the way that civil servants exercise discretion over the visits of foreign scholars, the way in which a "tide of conformity and fear" menaces learning in the public schools of the United States, and the way in which academic communities themselves are responsible for "assaults on intellectual freedoms that shame their traditions of liberty".

It is stirring stuff, but unfortunately true as well. The question Schmidt ducked at the weekend is whether, and if so how, present constraints on the freedom of universities and other academic institutions are avoidable, or curable. As with charity, the place to start is at home, which in Schmidt's case is a good place. Yale may have a reputation for turning out bankers, but there is nothing wrong in that, while the quality of its undergraduate education and of its research community are generally admired. If Yale has an identifiable fault, it is that its administration is rather more powerful relative to academic interests than it might be.

To the extent that the academic freedom of an academic institution must be a function of the degree to which its academics function as self-starting well-springs of academic policy, there is a case for making sure that, in the last resort, it is academics who call the tune. Most institutions need to be edged in this direction.

The fear that academic self-determination is a licence for academic indecision is, in reality, the opposite of the truth. Rights engender responsibilities, especially when the education of the young is a common objective. But Schmidt clearly believes that it is still possible for a university president to give his institution a sense of direction and thus a greater degree of independence. It will be interesting to see how his good speeches become reality. □

AIDS therapy

First tentative signs of therapeutic promise

Washington

THE US Public Health Service last week announced that azidothymidine, or AZT, is the first therapeutic agent to show promise in treating victims of AIDS (acquired immune deficiency syndrome). Phase II clinical trials have shown such encouraging results that the health service has modified the studies in mid-stream to enable the placebo control group to be offered the drug. Burroughs Wellcome, manufacturer of AZT, said it would make the drug available to "a certain narrow category of patients with AIDS", but no more than 6,000 individuals may fit the criteria. And hobbled by a world-wide shortage of thymidine, an essential ingredient of the drug, the company may be hard pressed to keep its pledge.

All those concerned are eager to point out that there is no evidence, as yet, that the drug is a cure for the disease. What the trials have shown so far is merely that it prolongs the lives of an important subgroup of those infected.

Data released by Burroughs Wellcome and the health service last Friday reveal that, in tests involving 282 patients, AIDS victims receiving AZT had "a significantly lower mortality rate than those receiving placebo". During the course of the six-month study, 16 deaths occurred in the placebo group and only one in the group receiving AZT, whose members also showed a lower incidence of lymphomas, Kaposi's sarcoma and opportunistic infection. They gained weight and showed some improvement of immune function. The Data Safety Monitoring Board last week decided that it would be unethical to continue to withhold the drug from the placebo group.

The government and corporate teams involved in AZT testing also agreed to extend investigational new drug treatment to all individuals belonging to the trial subgroup deriving the greatest benefit from the use of the drug during the clinical trials. This group consists of AIDS victims who have contracted *Pneumocystis carinii*, which accounts for about 60 per cent of the 11,000 living AIDS patients in the United States, according to Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases. People would be given the drug only on the recommendation of a licensed physician.

AZT acts by competitive inhibition of reverse transcriptase, the enzyme that converts the RNA of retroviruses such as that responsible for AIDS into DNA which, incorporated in the genome of the

infected cell, will ensure the replication of the virus. As a nucleoside analogue, AZT gets incorporated in the DNA strand where its unruly structure halts transcription and hence virus replication. There is evidence that AZT can prevent further deterioration of the immune system, and even allow some regeneration, according to Samuel Broder, deputy clinical director at the National Cancer Institute.

But, if AZT interferes with the replication of retroviral genomes, why does it not also interfere with the polymerase enzymes essential to normal cell function? Broder said polymerases are much less sensitive to the drug, and that this scenario might explain the bone marrow suppression observed in some patients taking AZT. Indeed, anaemia and low neutrophil and granulocyte counts are the most important side effects of AZT.

Because the phase II trials spanned only six months, little is yet known about the long-term effects such as toxicity, tolerance and sustained response. Nor is it clear that AIDS patients outside the group selected for the trials will reap the same benefits from the drug.

Even if further studies show that AZT is no more promising than in the first round of clinical trials, there may not be enough of it to go around. Thymidine, the primary raw material used in AZT synthesis, is usually extracted from herring sperm and is becoming more and more difficult to get, according to Burroughs Wellcome's head of research, David Barry. AZT synthesis takes 26 steps, several months and "many millions of dollars" to complete, Barry says. The company seems confident that it can meet the demand for treatments over the next few months, but it may be scrambling when the Food and Drug Administration approves AZT for more general release. Yet Broder thinks approval may come as early as December.

In the light of this potential shortage, many AIDS researchers are stressing the need to continue with tests on other drugs. They fear that reports of AZT's success are bound to make research initiatives more difficult.

In Britain, Burroughs Wellcome's parent Wellcome Research Foundation expects permission to begin controlled clinical studies next month, yet the company's supply of AZT in Britain is "tiny", according to David Langford, Wellcome's head of clinical immunology. Langford says the unexpected efficacy of AZT has "truncated" attempts to develop more efficient syntheses.

Karen Wright

Arms control

Stockholm talks succeed at last

THE agreement reached in Stockholm at the week-end is without precedent among arms control agreements, both in its provisions requiring advance notification of conventional troop movements and the detailed provisions for on-site inspection, demands for which may be made by any of the 35 signatories of the treaty. The agreement covers land forces only, but the territory between the Urals and the Atlantic.

The negotiations, which lasted close on three years, are an offshoot of the Helsinki accords of 1978, involving all European states (except Albania), the Soviet Union and the United States. The Helsinki documents required that the member states should explore technical schemes for building confidence and security in Europe. Progress at Stockholm, much of which has been squeezed into the past few months, seems to have been spurred by next month's deadline, when an account of the talks has to be given to a Conference on Security and Cooperation in Europe meeting in Vienna.

The chief provisions of the agreement are that all signatories should notify all others of military movements involving more than 13,000 people (or of 3,000 paratroopers or troops landing from amphibious craft). If the numbers of people involved exceed 17,000, observers from all other member states will be invited to attend to satisfy themselves that what is afoot cannot be counted threatening.

These numbers are approximately equal to but somewhat larger than the numerical strength of an army division, and have been the chief bone of contention at Stockholm in the past six months. Western powers had been seeking a number so small, perhaps as low as 8,000, that single Soviet divisions would be caught. But the numbers are much smaller than originally offered by the Soviet side.

All signatories of the treaty will be required to produce, on 15 November each year, an annual calendar of planned troop movements in the year ahead. Movements or concentrations of 40,000 troops will have to be notified at least a year in advance, while movements of more than 70,000 troops will have to be announced in the calendar two years in advance.

Apart from the obligation to invite observers to exercises involving more than 17,000 land troops, the treaty will also give member states the right to demand inspections of suspicious events (but no member will be required to host more than three inspections a year). These provisions importantly treat all members of the Warsaw Pact and the North Atlantic Treaty Organization on an equal footing. □

Japanese grants

Once-in-a-lifetime chances

Tokyo

BIOSCIENCE and optoelectronics have pulled in most of the biggest and best research grants in Japan this year, according to figures recently released by Japan's Ministry of Education Science and Culture. But the search for the elusive heavy neutrino also gets a significant boost.

The Ministry of Education, Science and Culture has over ten categories of grants-in-aid for scientific research, ranging from run-of-the-mill general grants of a few thousand pounds to special research grants that can command a cool million. Amongst the latter, the *crème de la crème* are the "special distinguished research" grants awarded to individuals for 3 to 5 years to carry out "internationally recognized research that is likely to produce outstanding results".

Applications for these prestigious grants are made in December each year. After an initial screening, about twenty researchers are summoned to the ministry in April to present their research plan before members of the Science Council. Of these about half are selected and the grants are awarded in July.

This year, bioscience comes out on top, taking five out of ten of this year's awards. For example, Tasuku Honjo of Kyoto University gets Y251 million (about £1.1 million) over five years to continue his pioneering research on immune diversity

and in particular interleukins, while Mutsuo Sekiguchi of Kyushu University School of Medicine receives Y227 million to investigate the regulation of DNA repair and mutagenesis with the aim of unravelling some of the mysteries of cancer and genetic disease.

Optoelectronics also figures prominently with two awards totalling over Y400 million. But the biggest grant of all, at Y268 million (about £1.2 million), goes to Hiroyasu Ejiri of Osaka University for a five-year study of neutrinos. In an attempt to determine the mass of the neutrino and the amount of right-handed current in the weak interaction, Ejiri's group will monitor the neutrino-less double beta decay of a 70 cm² piece of molybdenum foil in a mine-shaft 1,000 m underground in the middle of a mountain at Kamioka in Gifu Prefecture. The mountain will filter out cosmic radiation allowing the observation of perhaps ten events per year.

Research is not all that these awards entail. Grant holders are inundated with requests to write scientific articles, and as these often come via members of the Science Council, they are hard to refuse.

Another drawback of getting an award is that under the "democracy" of Japanese science one can never get another one. In many ways it pays to be a co-researcher rather than the named awardee.

David Swinbanks

Fairer grants system for 1987?

Tokyo

JAPAN'S Ministry of Education, Science and Culture is overhauling its system of "special" research grants in an attempt to make it more open, flexible and responsive to the needs of today. The new system will come into effect from fiscal year 1987.

At present the ministry has three categories of special grant, the special distinguished grants (see above), special long-term grants and special project grants. The special project grants are an unusual feature of Japanese science and allow participation of large numbers of researchers from various institutes under the banner of a single 3-year project. For example, Seturo Ebashi of Okazaki National Institute for Physiological Sciences this year heads a team of 38 from 20 universities investigating vascular function. Funding for the project this year amounts to Y190 million (about £830,000).

The special long-term grants currently cover four major fields: cancer, natural disasters, the environment, and energy and fusion. Each field receives annual funding of between about Y500 million and

Y2,000 million (£2-8 million) dispersed among 100-200 projects.

The special grants, however, came under criticism because the project titles and leaders were designated by the Science Council of Japan, an advisory body of 210 scientists from all disciplines, and only well connected scientists tended to be selected. Further, only those scientists personally acquainted with the project leader could hope to join the project.

Under the new system, the special long-term and special project grants will be partially merged into a new category called "priority areas of research", although cancer and fusion research will remain separate. Grants will run from 3 to 6 years with annual funding of Y50-800 million. But the major change is in the selection of the projects and participants.

Although a few priority areas will be designated by the ministry, most will be selected from proposals made by researchers throughout Japan. And once project titles are selected, any researcher, even high-school teachers, can apply to participate.

David Swinbanks

India's space programme

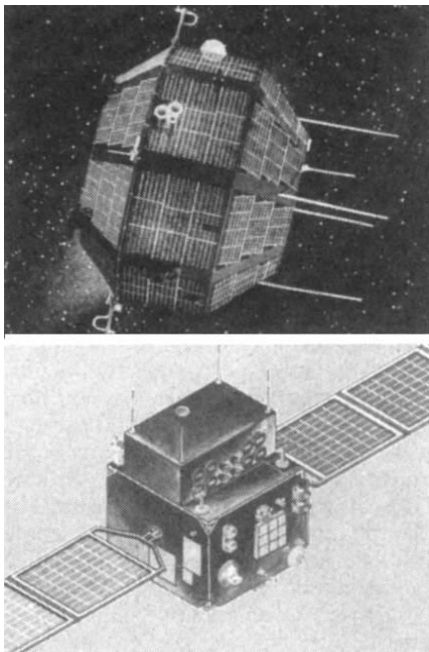
Rising above forest decline

Bangalore

ALARMING results from the latest Landsat imagery suggest that deforestation in India is occurring at a much greater rate than was previously feared. The Indian Forest Department had believed that forests covered some 22 per cent of the nation's land area, but Landsat reveals only 10 per cent cover. To get some measure of the extent of the problem the Indian Planning Commission's task force on resource management has recommended that satellite images should be used to compile a vegetation map of the country, and a new Indian satellite to be launched next year will be able to contribute to the mapping programme.

The two Bhaskara satellites, launched in 1979 and 1981, have provided Indian space scientists with experience of sensor design and management of the data received by ground stations. Next year the Indian Remote Sensing Satellite (IRS) is to be placed into a polar/Sun-synchronous orbit by a Soviet launcher. IRS, now being built in India, will weigh 850 kg and is a fully-fledged remote sensing satellite stabilized on three axes and featuring deployable solar panels. After IRS, a series of satellites with greater resolution and more channels is planned.

The resources task force also recommends the setting up of remote sensing "cells" in the forest regions of all states so that the information gathered can be distributed to forest workers.



Two steps in India's remote sensing programme. Top, Bhaskara-II, launched in 1981; bottom, an artist's idea of IRS.

Communications should benefit, too. Communications satellites are seen as the logical means of improving telephone and broadcasting services. The Indian Space Research Organization has now been granted the budget to start building its INSAT-II satellite at the Bangalore satellite centre. The first of the INSAT-II series is due to be launched in the early 1990s atop a new liquid oxygen/liquid hydrogen driven geostationary launch vehicle now being designed by Indian space scientists.

With INSAT-II in operation it should be possible to run as many as eight television channels. Each satellite will be able to carry 6,400 two-way telephone circuits.

Roof-top antennas will then lead to a great improvement in communications — particularly important for the development of business and for better governmental administration.

The immediate future of the Indian space programme, however, lies with the Augmented Satellite Launch Vehicle (ASLV). This solid-fuel rocket is to become the workhorse for the launches of the 150-kg "stretched" Rohini research satellites during the late 1980s. ASLV's maiden flight is due later this year and it is hoped that the second flight, in 1987, will launch a satellite carrying a remote-sensing payload built in West Germany.

Radhakrishna Rao

US drug tests

Hit-and-miss results are likely

Washington

APPROXIMATELY 1.1 million federal employees will have to submit to screening for illegal drug use under the terms of an executive order signed last week by President Reagan. But before the White House implements the new drug testing programme, some members of Congress want one question answered about the tests: do they work?

A first evaluation by the Office of Technology Assessment (OTA), the independent congressional office, has concluded that adequate technology exists to carry out a screening programme. But whether that technology will be used properly, and what will be done with the answers it provides, are still items for debate.

Lawrence Miike, a senior associate in OTA's health programme, last week presented the findings of a preliminary investigation on the accuracy and reliability of currently available urine drug tests to a House of Representatives subcommittee. The most common tests use either thin

layer chromatography, enzyme multiplied immunoassay or radioimmunoassay to screen for drugs. Test kits available to clinical laboratories contain reagents that permit testing for several drugs from one urine sample. The three methods vary in their sensitivity and specificity for different compounds, but all can cross-react with substances in urine that do not result from illegal drug use. Miike considers that all positive tests will need to be confirmed using more sensitive techniques.

But even the most accurate test is useless if it is improperly performed. Regulations governing laboratories vary widely from state to state, says Miike, and monitoring efforts are often ineffectual. A proficiency testing programme run by the Centers for Disease Control focused primarily on clinical testing rather than drug screening, but that comes to an end at the end of this month. Three professional societies offer proficiency testing programmes for urinary drug screening, but these are on a voluntary basis. The best

indication of expected performance from a large-scale screening programme comes from the military, which is now screening about 3 million people a year. Estimates suggest these tests are correct 90 per cent of the time, but as false negatives will typically go undiscovered, this figure is based only on false positive errors.

Miike raises several issues concerning a large-scale screening programme, which must necessarily give only a yes/no answer on drug use. For this reason, heavy users are classified with those with barely detectable levels of a particular drug.

I'm sorry, Mr. President—the machine says you're addicted to cheap publicity...



Determining the cut-off point therefore becomes critical for determining who will be caught in the screening net. As increased use of the tests will promote improvements, more sensitive tests will enable the cut-off point to be lowered; Miike questions whether technology should be the driving force in arriving at public policy on illegal drug use.

There is also the problem of what drugs to test for and what to do with the results. Analgesics, sedatives, tranquilizers and stimulants are sometimes abused, but all also have proper clinical applications. Different government agencies have already reached different answers to this question. A survey earlier this year by the Civil Service revealed wide variations in the drugs to be screened by different agencies. As an example, the Army intended to test only for marijuana and cocaine, while the Department of Treasury's Secret Service planned to test for amphetamines, barbiturates, cocaine, various pain killers, opiates, quinine, phencyclidine, methaqualone and tranquilizers (but not marijuana).

No wide-scale testing programme will be cheap. President Reagan estimates his programme will cost \$56 million. Representative Patricia Schroeder (Democrat, Colorado), puts that figure closer to \$300 million when costs relating to rehabilitation efforts and legal complications are taken into account. Miike says estimates of the direct cost of testing vary from \$15 to \$100 per person. But despite intrinsic limitations, Miike concludes that with appropriate confirmatory tests, a positive result is "highly reliable and difficult to dispute."

Joseph Palca

Example 1 :50 per cent

		DRUG IN URINE		
		Yes	No	
DRUG TEST	Positive	95	10	105
	Negative	5	90	95
		100	100	

Example 2 :10 per cent

		DRUG IN URINE		
		Yes	No	
DRUG TEST	Positive	19	18	37
	Negative	1	126	163
		20	180	

Mass screening programmes using tests that are even marginally inaccurate face a statistical problem depending on the actual frequency of the condition screened for. This figure presents a hypothetical example developed by Lawrence Miike of the Office of Technology Assessment. In this example, the test for drugs in urine has 95 per cent sensitivity, so for 100 positive samples 95 will be correctly identified, and there will be 5 false negatives. The test is also assumed to be 90 per cent specific, meaning that there will be ten false positives from 100 samples that contain no drug. But the test's predictive value varies considerably depending on the expected frequency of positive samples. In example 1, half of 200 urine samples are assumed to show drug contamination. There are 95 correct positive tests and 10 false positives, giving a predictive value for positive test results of 95/105 or 90.5 per cent. But if the expected incidence is only 10 per cent, or 20 out of 200 samples, the picture changes radically. Now there are 19 correct positive tests, but 18 incorrect ones. The predictive value of the positive tests in this situation would be only 51.4 per cent.

Stratospheric ozone

Global limit for CFC emissions

Washington

INTERNATIONAL support is growing for a global cap on chlorofluorocarbon (CFC) emissions in order to avoid potentially serious climatic changes. So much has emerged from an informal meeting two weeks ago of the signatories of the Vienna Convention on Protection of the Ozone Layer. The meeting reached broad agreement on the need for controls. This could lead to an international protocol as soon as next summer.

Quite apart from the protection of the ozone layer which such an agreement would provide, an international agreement to restrict the emission of particular materials could be a precedent for similar accords on the regulation of other global pollutants including carbon dioxide, the chief candidate as culprit in the greenhouse effect.

The Vienna convention, the first to tackle a potentially global environmental problem, was signed in March 1985 by 21 countries; four more have since signed. Signatories agreed to hold informal workshops to discuss technical issues before the opening of formal negotiations on a control protocol, which must start in Geneva in December. At the final workshop at Leesburg, Virginia, US Ambassador Richard E. Benedick said that "there seems to be agreement that the world has entered a danger zone" and that almost all countries consider the risks "sufficiently serious as to warrant control actions".

CFCs are widely used as refrigerants, as solvents and in aerosol sprays. Although it is still uncertain how soon environmental problems due to CFCs may appear, all agree that their reaction products contribute to the breakdown of the stratospheric ozone layer. They are also "greenhouse gases" that could contribute to global warming. The worst offenders are CFCs 11 and 12; substitutes are available for many uses but often have disadvantages such as flammability and toxicity.

In 1985 an international study concluded, on the basis of two-dimensional atmospheric models, that continued releases of CFCs 11 and 12 at 1980 rates would reduce the ozone vertical column by a global average of 9 per cent, with reductions of up to 14 per cent in polar regions. But predictions are made difficult by the close coupling of CFC stratospheric chemistry and that of other pollutants. Observations of actual decreases in ozone have been hard to interpret, but there does seem to have been a 2 to 3 per cent decrease over the period 1970-80.

Agreement on a control protocol seems imminent after a productive workshop in Rome but not all is sweetness and light. US participants at Leesburg were

annoyed that the European Community and European industry still seem unwilling to contemplate further controls. The European Community has voluntarily adopted a cap on European CFC production capacity, but US critics point out that, with European production much less than the ceiling, production (and hence emissions) could continue to increase into next century before the cap would be effective.

In the United States, there has been a ban on the non-essential use of CFCs in aerosols since the 1970s and the US government has tried to persuade others to follow suit. US government sources hint that, if Europe does not now come into line, the substantial European export trade in CFCs could be affected.

Significantly, the Alliance for Responsible CFC policy, a US industry consor-



tium, has dropped its opposition to international controls, and now supports US attempts to negotiate an international protocol. The alliance has called for the US government to cooperate with other countries to fix a "reasonable global limit" for the growth of CFC production.

At Leesburg, the consensus was in favour of a cap on global emissions, rather than on the restriction of end uses. Much attention focused on a Canadian formula that would fix national quotas for CFC production as proportions of a global limit weighted 25 per cent for population size and 75 per cent for gross national product. British representatives at Leesburg (who were not formally representing their government's position) did not exclude the adoption of such a formula, but suggested that a change in the total that Europe could release at the same time might be politically too big a step in one year.

Observers were encouraged by signs of interest in global action from the Soviet Union, which for the first time gave figures on its CFC 11 and 12 production. Japan proposed a two-tier control system that would place immediate restraints on growth, followed by more controls as data become available with which to fix a safe emission limit.

Tim Beardsley

British research funds

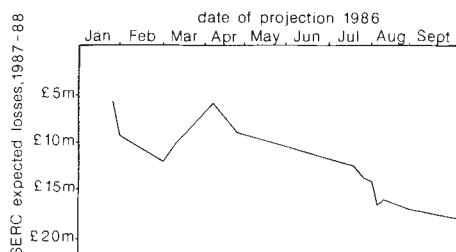
Pound's fall an embarrassment

THE recurring problem of foreign exchange once more threatens to beggar the Science and Engineering Research Council (SERC), Britain's principal source of basic science funding. According to the latest estimates (see figure) the council is likely to be a record £18 million in the red next year on international subscriptions of some £70 million, which make up nearly a quarter of its annual budget. This is the effect on British science of the falling oil price, which hit oil-rich Britain on the foreign exchange markets and pushed up SERC's international payments.

The council owes its international dues in Swiss francs (for the European Organisation for Nuclear Research, CERN), European Currency Units (for the European Space Agency, ESA), French francs, Belgian francs, Swedish kronor and Australian dollars. But the rub is that the British Treasury only pays the council in pounds sterling. In the now rare years when sterling is strengthening, the council can make a book profit on the exchange rate. But most of this must be paid back into Treasury coffers. Years of argument have resulted in agreement with the Treasury that the council can now save 4 per cent of its subscriptions (£0.7 million) from one year to the next, or shift up to £1 million of any exchange rate profits into domestic expenditure. But this facility is nowhere near enough to meet the projected crisis in 1987-88.

In the current financial year, corrections made at the time of the public expenditure review in October 1985 plus the "Christmas present" of the then Secretary of State for Education and Science, Sir Keith Joseph, means the council expects to break even. But the corrections came late in the day, and were unpredictable largesse. Moreover, they only amounted to £12 million, whereas a 50-per-cent larger correction seems necessary for next year. In its advice to government this summer, the Advisory Board for the Research Councils (which divides up the British government's basic science budget) recommended that SERC receive a £9 million adjustment for exchange rates, but even this is now out by a factor of two. Long-term research planning is thus made impossible, council officials complain.

In place of this uncertainty, the research council is seeking a new system of funding: a "variable cash-limited budget". This would be fixed partly in sterling, and partly in foreign currency. Thus the Treasury would suffer the exchange uncertainty, instead of "the whole thing being dumped on our plate", says SERC secretary Ashley Catterall. The Treasury, however,



The UK Science and Engineering Council's increasing haemorrhage from its 1987-88 budget, with respect to plans put before the Treasury early in 1986, as the falling pound pushed up the cost of foreign-currency subscriptions to international programmes. Losses are now touching £18 million.

insists it will stick to the cash-limits it understands, in sterling, and refuses to insulate its payees from the consequences of their folly in making commitments in foreign currencies. Council sources perhaps unsurprisingly describe the argument for variable cash-limited budgets as "not getting very far". CERN, whose subscription is the council's largest, shows little sympathy; the issue is seen as Britain's self-inflicted wound, and one that Britain must sort out.

In October, the council will be considering to what extent any shortfall will have to be covered by cuts in studentships, or in research. If there are cuts, they will probably be at the margins of SERC's activity,

officials hint darkly. Training activities not directly connected with research — such as the advanced course studentships, costing some £10 million or so annually — may be threatened.

Moreover, there could be even more than £18 million to find because another international problem is looming for SERC: the extent to which the Treasury will wish to 'attribute' grants received by British researchers from the growing European Commission research programme. Under the strict interpretation of Treasury rules, the British science budget could be cut to keep a fixed level of science funding in Britain. At present little such 'attribution' appears to take place, but if European Commission research budgets increase dramatically next year (and 50 per cent is not unlikely), the Treasury seems likely to wake up to the possibilities and act.

This, however, would be seen as another self-inflicted wound. British scientists are highly successful at winning European funding, and certainly receive back more than the proportionate British subscription to the European Community. How much of the present European funding in Britain might be attributable to SERC? Dr Catterall considers it not in the council's interest even to "guesstimate". But the figure is likely to be in the region of millions of pounds. **Robert Walgate**

Biology is the soft option in Albania

YOUNG Albanians are showing a distressing tendency to try to use higher education as a way of avoiding "productive labour", the youth daily *Zeri i Rinise* complained recently. Instead of becoming "engineers, geologists and builders", they wish, the paper says, to become "lawyers, philosophers and biologists". The inclusion of biologists in the list is in marked contrast to other socialist countries, where biology is definitely production-orientated. (Do the scars of Lysenkoism linger yet, one wonders, in Stalinist Albania?)

This "petit bourgeois" mentality, the paper notes, is particularly reprehensible in that it affects workers and peasants, who want their children to find non-productive "intellectual jobs". It is understandable, *Zeri i Rinise* notes sourly, if the children of doctors feel that they too should like to study medicine. But toilers in factory and field, it implies, should encourage their offspring to follow in their own, proletarian footsteps.

The issue is particularly important since Albanian university admission procedures are heavily weighted in favour of the children of workers and peasants — with the unspoken assumption that, in due course, they will return, with their new-won skills, to the ranks of the toilers. Nor is this weighting a merely symbolic one. According to the vice-rector of Tirana University, Hasan Mucostepa, this means that during the academic year 1985-86, some 50 per cent of all students at the university (Albania's principal higher educational institution) were the children of workers and peasants. **Vera Rich**

suited for large-scale power production and excel in space, while direct solar panels of his own type are ideal in inaccessible places.

Green's team is now attempting to apply the lessons learned in the development of last year's cell to the world of mass production, where the high purity of the materials used will not be easily repeated. The work is supported by BP (British Petroleum) Solar Australia and the Sandia National Laboratories on behalf of the US Department of Energy.

Green reckons that his group is approaching a good compromise between cost and efficiency with a solar cell offering 18.8 per cent efficiency with a silicon wafer taken from BP Solar's production line. One important innovation has been the use of copper rather than silver contacts, which are embedded in slots cut by laser beams, well suited to mass production. It remains to be seen how best to scale up from the 2 cm square cells used last October to the 10 cm disks normally used. **Charles Morgan**

Solar power

Australia joins the solar race

Canberra

AN Australian team at the University of New South Wales, Sydney, reckons to be racing neck and neck with US laboratories towards the goal of an efficient silicon solar cell. But interest is now turning from the search for ever greater theoretical efficiency to the design of cells that will be economic in the field.

Australia has long been in the forefront of solar cell research, partly because of its latitude and its often cloudless skies but principally because of the remoteness of many of its communities. In the late 1970s, Australia was the largest market for solar cell applications.

Much of the recent running has been made by a group led by Dr Martin Green, itself part of the Microelectronics Research Centre at the university, which has been singled out as one of ten centres of excellence by the federal government in Canberra. Last October, Green's group announced the design of a silicon cell with a conversion efficiency of just over 20 per cent, then the highest ever attained.

The essence of the Australian development is to etch 10 µm microgrooves into the surface of a silicon wafer, which increases the efficiency of solar photon collection by internal reflection and reduces

the chance of recombination between conduction electrons and electron holes by concentrating the formation of electron-hole pairs near the N-type silicon layer on the surface of the grooved structure. The grooves also increase the range-section area available for conduction by a factor of 3, reducing the resistance of the cell.

Last year's record efficiency was obtained with a grooved cell coated with a thin layer of oxide, which appears to reduce lattice defects. Steps were also taken to reduce the distortion caused by the attachment of electrodes to the cell. Even so, the Australian record was eclipsed earlier this year, when a group at Stanford University in California announced the development of a cell with a conversion efficiency of 27.5 per cent, but only with the use of solar radiation concentrated on the active surface by a system of Sun-tracking Fresnel lenses.

Comparisons are difficult. Cells such as those developed at Stanford require steering systems, and can use only the direct component of solar radiation (on average, three-quarters of the total) while the costs are effectively determined by the Fresnel lenses. Green says that the two types of systems are at present roughly on a par. Concentrating solar cells are best

Chemical warfare

United States still ill-prepared

Washington

A GENERAL Accounting Office (GAO) report* lambasting the US Defense Department's efforts to improve its defensive capabilities for chemical warfare has caused a ripple in Washington. The report, delivered to Congress at the end of July and made public this month, finds that although there have been improvements in the military's defensive capabilities and ability to fight on a chemical battlefield, areas of medical doctrine and personnel training are severely deficient.

GAO initially set out to determine the adequacy of the Pentagon's preparations



Chemical defence USAF-style

for chemical warfare. But in many cases, the report contends that the Defense Department had established no criteria for determining adequacy, so the GAO changed its focus to how far the Pentagon had come in its preparations since 1982.

There has been progress in getting defensive equipment into the field, but many problems remain. Currently the Defense Department has no antidotes against blood, blister or choking agents. Pyridostigmine, a British drug thought to be effective against all nerve agents, is just being introduced into the field. Problems continue to plague efforts to design effective protective garments. An adequate face mask has yet to be developed, and the latest suit to be used by the Navy "loses its protective qualities when it is wet". GAO has also identified problems in plans for decontaminating equipment following attack, detection of attack and availability of chemical defence equipment.

Although the Defense Department has had an active programme of research on chemical warfare defences, the GAO says the programme has been marked by "numerous delays and cancellations", and relatively few items have actually been put into the field. Funding shortages cannot

be blamed.

Training procedures for using existing equipment are also inadequate, according to the GAO report. Although there has been improvement in chemical warfare training, without more frequent and realistic testing it is impossible to evaluate the military's state of readiness.

Defense Department spokesman Major Randy Morger says the Pentagon "agrees with the bottom line" of the GAO report. Morger says thousands of millions of dollars could be poured into chemical defences, but no fighting force will be truly effective using defence measures alone. Morger acknowledges that GAO has

pointed out some problem areas, and there may even be others that GAO has missed. But he argues that an effective retaliatory capability is necessary to deter chemical attack.

While the Pentagon appears willing to take criticism for its defensive efforts, it was not so sanguine about suggestions that the centrepiece of its new offensive capability, the Bigeye bomb, might be flawed (see *Nature* 321, 717; 1986). The Pentagon took great pains to refute criticisms of Bigeye. But Eleanor Chelmsky, director of GAO's division of programme evaluation and methodology, says the defensive programme is "really in bad shape, but it doesn't seem to upset anyone."

Joseph Palca

*Chemical Warfare: Progress and Problems in Defensive Capabilities. GAO/PEMD-86-11. Washington, DC 1986.

Biotechnology patents

New rash of legal suits ahead

Washington

CETUS and Genentech, two of the most venerable biotechnology companies in the United States, are bracing themselves against separate patent challenges raised this month over some of their most promising products. Disputes have erupted between Cetus and Amgen over several forms of interleukin-2 while Hoffman-LaRoche, with the California-based Hormone Research Foundation, is contesting Genentech's claim to recombinant human growth hormone.

These suits are among the first generation of patent battles in biotechnology and, as such, will have few precedents to guide them. But an appeals court's decision last week to restore patent privileges to Hybritech for the sandwich assay protocol seems to betoken fair wind for the patent-holder, foul for the challenger.

In a move akin to a pre-emptive strike, Amgen announced late last month that it was seeking a ruling that it is not violating three of Cetus's four interleukin-2 patents. Although a Cetus spokesperson declares that, before Amgen's action, the company "had no intention of bringing a lawsuit against Amgen", Cetus joined the fray two weeks later claiming infringement. Part of the controversy concerns a form of interleukin-2 sold by Amgen which Cetus claims is identical to one of its own patented interleukins.

Even more heat surrounds the pure forms of interleukin-2 Cetus is producing for clinical trials conducted by the National Institutes of Health. Johnson & Johnson and Hoffman-LaRoche, both in New Jersey, are trailing with their own trials, and Amgen supplies a very pure form of interleukin-2 to Johnson & Johnson. Cetus does not know the composition of Amgen's product, but believes that its two patents on purified interleukin en-

compass any product pure enough to meet Food and Drug Administration standards for clinical trials. The company will not comment on its position with regards to Roche.

Roche, meanwhile, has been trying to wrest a licensing agreement from Genentech for human growth hormone. Roche, not Genentech, claims to possess the original patent on "synthetic human growth-promoting . . . hormone," issued in 1974 to C.H. Li, a University of California scientist who performed the first organic synthesis. Li filed his patent two years before Cohen and Boyer patented the notion that bacterial plasmids might be engineered to generate specific proteins; he formed the Hormone Research Foundation to further his studies.

Hoffman-LaRoche gained exclusive rights to Li's patent in 1982, hoping to capitalize on the fruits of recombinant work on growth hormone by signing licensing arrangements with the purveyors. Genentech, however, has balked at this strategy on the grounds that "synthetic" does not include, and could not have anticipated, recombinant-DNA products. Two weeks ago, Genentech, which worked closely with Roche on alpha-interferon, announced its intention to defend its position.

Both disputes will take years and millions of dollars to resolve and, according to one industry analyst, are just the first blush of a burgeoning harvest. Iver Cooper, author of *Biotechnology and the Law*, thinks a dozen such lawsuits will be pending by the end of the decade. And since Hybritech's appeal proved victorious, patent-holders may be willing to press cases beyond the first ruling. The scope and interpretation of patent law on recombinant-DNA products will have to be defined amidst the crossfire. **Karen Wright**

Crafoord prize

Isotope pair rewarded

Two isotope geochemists, Professor Claude Allègre of the Institut de Physique du Globe in Paris and Professor Gerald Wasserburg of Caltech, have won this year's Crafoord prize, awarded by the Royal Swedish Academy of Sciences. This younger relation of the Nobel prize has been given annually since 1980, to honour outstanding basic research in the "non-Nobel" fields of mathematics, astronomy, life sciences and earth sciences.

Isotopic ratios, which were first measured in rocks and minerals to determine their ages, are now used as sensitive tracers of geological and cosmological processes. Both prizewinners are skilled experimentalists who have developed highly



Allègre (left), mantle evolutionist, and Wasserburg, Solar System chronologist.

precise analytical techniques and applied them to important problems in the evolution of the Earth and Solar System.

Professor Wasserburg is perhaps best known for his work in establishing the chronology of the Solar System, which involved the analysis of very small lunar and meteoritic samples. Although he refers to his laboratory as the "Lunatic Asylum", he has also worked on terrestrial problems, using the rare gases and strontium and neodymium isotopes as tracers in systems ranging from the Earth's mantle to the oceans. Recently he has been using the isotope systematics of cherts, modern and ancient, as clues to the chemical evolution of sea water.

Although Professor Allègre has also worked on meteorite and lunar chronology, including making the first measurements of osmium isotopes using secondary ion mass spectrometry, his overwhelming interest has been in "chemical geodynamics" — the use of isotopes and trace elements as tracers of geological processes. His measurements of Sr, Nd and Pb isotopes in crustal and mantle-derived rocks have formed the basis for models of mantle evolution and the formation of the continental crust — models which he has recently been constraining using rare-gas isotopes in mantle-derived rocks.

In addition to the prize of 1 million kroner (£100,000), awarded to the two men, the Crafoord Foundation will give grants of 625,000 kroner for Swedish research in isotope geology. **Laura Garwin**

Lasker prizes

Threesome gain AIDS award

Washington

THE winners of this year's Albert Lasker Awards were announced earlier this week in New York. A total of six individuals were honoured; three for work on AIDS (acquired immune deficiency syndrome), two for the discovery of growth factors, and one for his contributions to public health in China.

The award for clinical medical research went to Myron (Max) Essex of the Harvard School of Public Health in Cambridge, Robert Gallo of the National Cancer Institute in Bethesda, and Luc Montagnier of the Pasteur Institute in Paris for "unique contributions to understanding of AIDS". The award comes in the midst of legal wrangling between the US National Institutes of Health and the Pasteur Institute over the discovery of the virus causing AIDS. A US patent for a blood test to screen for antibodies to the AIDS virus was awarded initially to Gallo, but may ultimately revert to Montagnier and the Pasteur Institute pending a patent office investigation. There has been an intense and occasionally bitter rivalry between the French and US investigators concerning research on the AIDS virus.

By awarding the prize to all three men, the Lasker jury sought to cast oil on troubled waters. But the waters are apparently still rolling.

A last minute change in the press announcement of the award credited Montagnier with discovering a virus "later shown to be the cause of AIDS", whereas the first version said his discovery was "the retrovirus responsible for causing AIDS". The change was made after US jurors raised objections to the original wording.

The award in basic science went to Rita Levi-Montalcini of the Institute of Cell Biology in Rome and Stanley Cohen of Vanderbilt University School of Medicine in Nashville for their work on growth factors. Levi-Montalcini is credited with discovering nerve growth factor, something she and Cohen both worked on at Washington University during the 1950s. Cohen later isolated and purified epidermal growth factor.

The award for public service went to Dr Ma Haide, senior advisor to the Ministry of Health of the People's Republic of China. His award is for the programme he

instituted that controlled venereal disease among China's 500 million population in the 1950s. Born George Hatem in 1910 in Buffalo, New York, Dr Ma travelled to China in 1933 to study tropical medicine. When war broke out, he joined Mao ZeDong's forces in the north. After the war, he stayed on in China, attacking the problem of venereal disease which was pandemic in China at the time. Dr Ma trained a small army of so-called barefoot doctors who went throughout the country finding venereal disease cases and treating patients with new antibacterial drugs rarely seen in China until then. The eradication programme was declared a success by 1959.

In the 1960s Dr Ma turned his attention to an effort to eliminate leprosy in China, and in 1983 he formed the China Leprosy Foundation.

Dr Henry Heimlich, professor of advanced clinical sciences at Xavier University in Cincinnati and himself the recipient of a Lasker Public Service Award in 1984 nominated Dr Ma for the Award. Heimlich describes Ma's accomplishments in controlling disease in China as "overwhelming". Dr Ma resides in Beijing where he has lived for 53 years. He is the first non-Chinese to hold a Chinese passport.



The Lasker winners. Top, left to right: AIDS workers, in alphabetical order: Essex, Gallo and Montagnier. Bottom: Cohen and Levi-Montalcini (growth factors) and Ma Haide (Chinese medicine).

Winners of the Lasker award receive a \$15,000 prize. The award, given since 1944, has forty-two times been a precursor of a subsequent Nobel prize. This is Gallo's second Lasker Award. He won the basic science award in 1982, along with four others, for contributions to the understanding of how normal cells are transformed into cancer cells. But no Nobel prizes have yet followed.

Joseph Palca

Anti-apartheid protestors safe?

SIR—Masters, Caithness and Rayner (*Nature* 320, 480; 1986) have proposed that there be a political-philosophical test on academics from South African institutions who wish to publish in international journals or to be admitted to conferences overseas. Their letter is a *cri de couer* reflecting the anguish, uncertainty and frustration of many scientists in South Africa who strongly oppose apartheid and look forward to a non-racial, democratic future, but whose dedication to the advancement of science and the freedom of movement of scientists now finds itself challenged by a growing tendency of countries and communities of scholars to exclude investigators resident in South Africa (as exemplified by the Southampton archaeology congress affair).

The acceptability of the proposal that a political test be applied before a scientist from a South African institution is admitted to an international congress is a subject for debate. Suffice it to say that the proposal is plainly at variance with policies on admission to international congresses maintained by two of the leading umbrella organizations of learned unions, the International Council of Scientific Unions and the International Council for Philosophy and Humanistic Studies.

But two points in the letter of Masters *et al.* may be misleading. First they state that "most funding for research (in South Africa) stems directly from the government". This is not strictly correct. It stems *indirectly* from government; as in Great Britain and the United States, statutory granting bodies are interposed between government and applicants for finance. Masters *et al.* then raise the possibility that congress participants, after signing an anti-apartheid document, might be "deprived of the financial support that should reasonably be theirs". This could be taken as a hint that the source of funding (commonly, the Council for Scientific and Industrial Research (CSIR), Foundation for Research Development, Medical Research Council (MRC) or Human Sciences Research Council) might penalize a researcher who signed an anti-apartheid declaration by refusing financial support.

I believe such a supposition is unjustified. All the evidence available to me from the past history, especially of the CSIR and MRC, the two bodies with which I have had most dealings over the years, would discount any likelihood that they would lend themselves to such an action.

Ever since Field Marshal J.C. Smuts set up the CSIR, under the presidency of Professor (later Sir) Basil Schonland, some forty years ago, the CSIR has been, to my knowledge, impeccably impartial in its disbursement of grants. For instance,

despite the well known stand of the Universities of Cape Town and the Witwatersrand against apartheid since 1948, scientists at these two universities consistently received most generous funding from the CSIR. Such grants went even to scholars who were outspoken public critics of apartheid. Similar comments apply to those at universities such as Natal and Rhodes.

The same policy has characterized the MRC since its inception in 1969 under Professor A.J. Brink. I know of no evidence of political discrimination, in decisions on research grants, against those whose philosophical convictions opposed those of the government of the day.

Hence, I feel there is no basis in past experience for the veiled inference of Masters *et al.* that the CSIR, the MRC, or any other South African funding body might discriminate in the future against scholars who have signed an anti-apartheid document such as they have proposed.

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Problems of Africa

SIR—Can Michael Spencer (*Nature* 322, 10; 1986) substantiate his statement that "Britain fostered tribal rivalries in colonial days by recognizing the Kabaka of Buganda as ruler" of Uganda? My experience of colonial Uganda and Kenya was that the colonial administrators fostered tribal identity and development rather than trying to form a unitary state. The Kabaka was recognized as head of state of the country of Uganda (rather than of the Baganda and the country of Buganda) only on independence. In colonial times, he was head of one of the four provinces of Uganda — Buganda, Western (including the kingdoms of Bunyoro, Toro and Ankole), Northern and Eastern. As to Museveni's popularity, it is not as widely shared as one might wish, with half the population from the other tribal groupings.

Whether Britain has squandered its oil wealth is surely not relevant to whether Nigeria has done so. Alberta has not, and Nigeria might have taken Alberta as a sound model, but Alberta is also in economic trouble. Nevertheless, the fact that Nigeria has not conserved its oil wealth or used it as development capital has contributed to its present economic woes.

Tanzania's falling income is due not only to falling prices of tea, coffee, sisal and other commodities but also to its systematic inability to maintain these agricultural industries. None of these commodities is easily available in Tanzania (as of March

1986) and even pepper and cloves are unobtainable in Arusha and other centres. Tanzania's economic woes are confirmed by its mismanagement of its foreign exchange, its insistence on an artificial and unrealistic exchange rate for its shillings at about 16 to the US dollar, when one shilling is really worth about two cents. The free market has caused part of the problem, but inability to allow for free market fluctuations, to keep the commodities being produced, to offer fair returns to the growers and to get the commodities to markets are basic to Tanzania's problem. In other words, it is a managerial problem.

As for the International Fund for Agricultural Development, that will succeed only if the population explosion in Africa is restrained, otherwise water and food will remain limiting and famine and disease will reduce many populations.

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Art of the "possible"

SIR—In "What to believe about miracles" (*Nature* 322, 321; 1986), R.J. Berry starts off on the wrong foot. His conception of a law of nature, and hence of a miraculous overriding of such a law, is far too weak. For if such laws were, as he maintains, "only generalizations of our experience", then a miracle, as a counter-example to such a brute fact generalization, would be at most merely improbable. It is only and precisely because laws of nature assert much more than this that the occurrence of a genuine miracle would be evidence of divine intervention, an overriding of the natural order by a supernatural power.

Berry and his friends are, of course, absolutely right in their insistence that the occurrence of miracles is, in this understanding, possible. Or, rather, it is possible so long as "possible" is taken to mean conceivable or logically possible. What it is not is physically or naturally possible. For anything inconsistent with a true law of nature must be, by definition, impossible, in this understanding of impossible.

When Hume's once notorious argument is so amended as to admit this familiar kind of impossibility — something which Hume himself, like Berry, failed to recognize — it becomes a formidable demonstration: not of the inconceivability of the miraculous but of the extreme difficulty, to put it no stronger, of knowing, upon purely historical evidence, that any miracles have actually occurred. (I have myself attempted to supply such amendment: see my *Hume: Philosopher of Moral Science*; Blackwell, Oxford, 1986.)

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Is megasequencing madness?

While there is a strong case for sequencing the entire human genome it should not be at the expense of other research. And sociological factors might yet impede the project.

HUMAN biology has entered a phase where no aspect is, or need be, left untouched by the techniques of molecular genetics. DNA sequencing is set to become a stock-in-trade for all investigators of human physiology or pathology. So the notion has arisen that the study of human biology would benefit enormously were the complete DNA sequence of the human genome to be available. With 3×10^9 base pairs to sequence, the task is formidable. But it is not impossible, given on a conservative estimate, 300 people, 20 years and a thousand million dollars. The question is whether the effort is worth the consumption of such resources. After a period of initial enthusiasm, many molecular biologists have backed off supporting the project in its entirety but not so Professor Walter Gilbert of Harvard University. Speaking at *Nature's* conference on Exploring the Human Genome, held in Boston last week, Gilbert argued against half measures or delay.

Gilbert points out that the number of base pairs that an individual could be expected to sequence in a year need only increase by 1 or 2 orders of magnitude to between 10^6 and 10^7 for 150 scientists (with 150 support staff) to sequence the entire genome in the course of 20 years. In fact, of course, machines would replace people for much of this work. Indeed, the first automated DNA sequencer will soon be available, and able to handle 10^6 bases a year, according to Leroy Hood, in whose Caltech laboratory the prototype was built.

In the course of the conference Hood voiced a frequently heard view that it would be wiser to spend a few years on developing the technology of automated DNA sequencing and the data processing systems that will be needed to handle the emerging information than to dive right into "megasequencing". To which Gilbert reasonably countered that the project of sequencing the entire human genome would serve to drive the development of the technology, so should be undertaken from the start. In time scale, the outcome of the two approaches is unlikely to differ since even Gilbert estimates that only 1 per cent of the sequence would emerge from the efforts of the first third of the project, with the last third accounting for 90 per cent of the sequence.

Acknowledging that all would not be plain sailing, Gilbert nonetheless made light of problems that, for others, have seemed a serious barrier to completing the

sequence. First, there is the problem that arises from the presence in the human genome of a considerable number of sequences that are repeated, sometimes in very many copies and sometimes in tandem. These could create confusion, if not havoc, in attempts to fit together randomly sequenced fragments of the genome. But, says Gilbert, they would present little trouble if the fragments are not random but of known physical relationship to one another, as he proposes. As to the problem of those few sequences that are bound to defy being cloned, for reasons that are not apparent, Gilbert takes the simple view that it does not matter if the "complete" sequence is slightly less than complete. More of a problem, perhaps, is the accuracy of sequencing; one error in a protein-coding sequence can make nonsense out of sense. For manual sequencing, errors are usually the result of carelessness and it will take much development before machines are even as good as careless humans. Ten years ago sequencers made about one mistake in 10^4 bases. Now, said Gilbert, it is more like one in 10^6 , a figure he was unable to sustain on questioning.

Set completely apart from these technical snags, whatever their size, is the problem of resources. In that respect, opponents of the complete project have some powerful arguments. One is that it would soak up all the most appropriate technologists, which is likely to be true but not a problem if there is widespread commitment to the project. Another is that the biological science community cannot afford to have diverted from other research the sum of \$30 million a year. Gilbert deflects the problem by saying that the project is not one of research but of the creation of a research tool. It should be considered along the lines of an industrial project both in scale and in the emphasis on quality control. The implicit message is that the project should not vie with genuine research for financial support but should be separately financed. There is no doubt that such a solution would be welcome, and with both the US Department of Energy and the Howard Hughes Institute already involved in discussions, the prospects of finding additional funds seem fair.

Particularly if financial support is to come from such sources, it is probable that it will go to a newly-created centre rather than being dispersed among several existing institutes. Gilbert argues for the centralization of the project in terms of

the economy of scale and the efficiency of the operation and if the idea of the project in its entirety is to be embraced, the case for a single centre is powerful.

But should the entire project be embraced? The alternative, which is gaining support, is that it should be much less ambitious, going only as far as the reduction of the genome into a collection of defined fragments of known physical relationship to each other and stopping short of sequencing them. Instead, the fragments of cloned DNA would be freely available to anyone interested in sequencing them for their own specific purposes. In that way, it is argued, the cost and scale of the operation would be contained and yet the outcome would be an invaluable starting point for an eventual sequence of the entire genome.

This has its attractions as a counter proposal. Chief among them is that the sequencing would be carried out with forethought and some expectations, rather than blindly. For example, particular fragments would be sequenced with the firm expectation, based on genetic evidence, that they contained genes that are responsible for heritable diseases. In that way, also, priorities for sequencing would automatically emerge so that the most important parts of the genome, at least by the self-serving definition that they are the parts that are currently receiving attention, would be sequenced first.

The inevitable price to be paid for such an approach is that each laboratory will need its own sequencing team and machinery, and will have to spend time in training technical staff. By the same token, time will be wasted in that competing laboratories will frequently sequence the same fragments. But at that point the problem becomes one of sociology. Can the major molecular biology laboratories agree to a joint effort in sequencing with, perhaps, little say in the operation? Can they give up their psychological addiction to sequencing? Gilbert says that the central purpose of a project designed to sequence the entire human genome is to provide a reference sequence against which variation can be measured by individual laboratories (and in that sense it matters not a jot whose genome is chosen). If somebody will pay for the project it has much to recommend it. But even if the money is found, a host of sociological factors may yet impede biology's entry into "big science".

Peter Newmark

Pharmacology

Virtuoso design of drugs

from Solomon H. Snyder

THE appreciation that many peptides are neurotransmitters has important implications. Some of the major neuropeptides turn out to be substances that are well known as hormones, a good example being cholecystokinin (CCK), an intestinal hormone that causes the gall bladder to contract, delivering bile to the intestinal lumen to facilitate digestion¹. Because of the importance of neuropeptides for body function, drugs that mimic or block their effects at receptors should be important as scientific probes and as therapeutic agents. Developing effective drugs to influence peptide systems is extremely difficult. The recent report^{2,3} by chemists at the Merck Sharpe and Dohme research laboratories of the synthesis of extremely potent, orally effective, non-peptide antagonists of CCK is a significant breakthrough.

About 100 neurotransmitter and hormonal peptides are now known to have physiological roles in many organs including kidneys, heart, gonads, pancreas, liver, lungs and brain. Each of these peptides is at least as important as better-characterized nonpeptide transmitters such as acetylcholine, histamine, dopamine and noradrenaline. Drugs have helped to elucidate the mechanism of some of the nonpeptide transmitters: for instance, cholinergic antagonists such as atropine clarify the action of acetylcholine; and amphetamines, which release the catecholamines noradrenaline and dopamine, are valuable probes. Enkephalins are one example of a neurotransmitter group whose physiological functions became apparent the day their structure was elucidated because opiates, drugs that mimic enkephalins, were already well characterized.

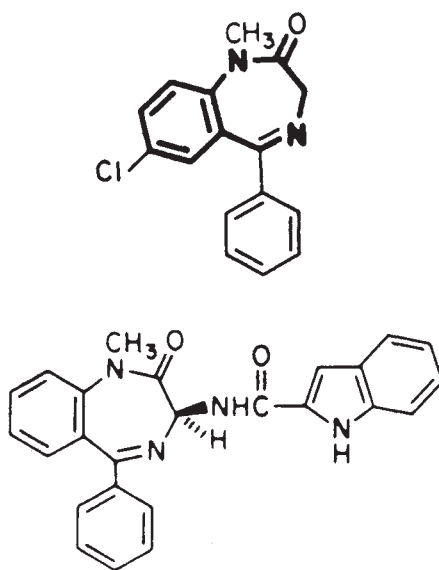
Drugs that influence neurotransmitters are useful therapeutically as antidepressants, antischizophrenic agents and anti-anxiety drugs, all of which act on a few, well-known, classical neurotransmitters. Drugs are not yet available that act selectively on the major neuropeptides, largely because discovery of these substances is so recent that the pharmaceutical industry has not had time to develop them.

Another reason for the lag in developing peptide-related drugs is that peptides themselves are poor drugs. An ideal drug should be active when administered orally, readily absorbed from the intestines into the circulation and should penetrate quickly into the target organs. Most biologically active peptides are rapidly degraded by the peptidases that normally digest dietary protein and it is difficult to synthesize a peptide that is readily ab-

sorbed from the gastrointestinal tract or to design a peptide that will readily penetrate the blood-brain barrier.

As a target for drug development, the Merck team focused on CCK because of its many physiological roles. CCK can be isolated from intestinal extracts⁴ in a 33-amino-acid form (CCK-33). Several smaller derivatives exist, with CCK-8 the major form in the brain⁵. CCK is used to contract the gall bladder and to image gall stones.

Within the brain the most prominent collections of CCK neuronal cells occur in the periaqueductal grey, an area where electrical stimulation produces analgesia, and thus a CCK antagonist might be ex-



The cholecystokinin antagonist L-364,718 (bottom) and the structurally related anti-anxiety drug benzodiazepine, diazepam (top).

pected to be an analgesic. Drugs based on CCK might also be relevant in the treatment of schizophrenia: the major antischizophrenic drugs act by blocking dopamine receptors. Their therapeutic effects are thought to involve blockade of the dopamine projections to the emotion-regulating limbic system, whereas the parkinsonian-like side-effects are caused by blockade of the dopamine pathway that passes from the substantia nigra to the corpus striatum. CCK is stored together with dopamine in the limbic dopamine projections but not in the nigrostriatal pathway⁶. If CCK and dopamine act as co-transmitters, a CCK-related drug could relieve schizophrenic symptoms without causing parkinsonian side-effects.

The search for a CCK-related agent took advantage of efficient strategies for

drug development based on receptor assays in which radioactive ligands bind to tissue membranes⁷. Receptor-binding technology is simple and precise, so about 1,000 samples per day can be assayed. Test chemicals can be selected at random that have no obvious structural relationship to the hormone or neurotransmitter under study. For peptide receptors, the search is not confined to peptide structures but can include conventional organic chemical molecules. Once a promising candidate emerges from the screening process, the molecule can be systematically varied and the derivatives screened at receptor sites. Successive iterations permit a swift escalation of molar potency.

The Merck scientists' search for CCK-related drugs began by screening of fungal fermentation broths. They identified a novel substance, asperlicin, which blocks CCK receptors in the 10^{-6} M concentration range⁸. Asperlicin possesses an indole moiety that might mimic the tryptophan of CCK, but otherwise it has no obvious similarity to CCK. A portion of asperlicin resembles the well-known anti-anxiety benzodiazepine structure, and because benzodiazepine chemistry is readily exploited, the Merck group focused on benzodiazepines linked to indoles. Its final product, L-364,718 (see figure), is more than 1,000 times more potent than asperlicin at CCK-receptor binding sites, blocking the ability of CCK to elicit contractions of the intestine and gall bladder. In mice the drug is orally active at doses as low as 0.04 mg kg^{-1} in preventing CCK-induced gastric emptying, and extrapolating these doses to humans, L-364,718 could be administered in doses of only 1 or 2 mg per individual, making it very potent.

The accomplishment of the Merck group is a landmark for several reasons. Most importantly, the work demonstrates the feasibility of using receptor technology to develop an orally active, nonpeptide drug that influences peptide receptors. In principle, similar success should be possible with many other neuropeptides. L-364,718 will provide a probe to elucidate CCK functioning: initial studies of CCK receptor-binding sites showed differences in peptide specificity between the pancreas and the brain, suggesting the existence of receptor subtypes⁹. L-364,718 is about 5,000 times more potent in the pancreas and gall bladder than in the brain, establishing definitively that central and peripheral CCK receptors have distinct

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recognition sites. Because of its selectivity for peripheral CCK receptors, L-364, 718 is unlikely to be useful in treating brain disorders, even though it probably penetrates the blood-brain barrier.

What might be therapeutic targets for a peripheral CCK antagonist? CCK itself decreases appetite both in animals and humans¹⁰, and some pharmaceutical companies have looked for CCK-mimicking

drugs as appetite suppressants. Whether L-364,718 and related agents will treat disorders of the stomach, intestine, pancreas, gall bladder or other peripheral organs is an open question. □

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Five-fold symmetry

Disorder among the atoms

from Alan L. Mackay

THE revolution in crystallography following the discovery¹ that textures with icosahedral symmetry can form from rapidly solidified alloys such as Al₃Mn is now at the stage where the new insights are being consolidated (see, for example, ref. 2). Knowles and Stobbs, whose latest work is reported on page 313 of this issue³, and others are trying to allocate positions to the atoms to account quantitatively for the electron, X-ray and neutron diffraction data. The task turns out not to be so easy for various reasons.

About six different alloys have so far been shown to have icosahedral phases, each of which is probably related by slight atomic movements to different crystal structures, so that no single arrangement of atoms can answer all the questions. There is also a phase known as the T-phase, which seems to be a periodic stacking of quasi-crystal sheets. Most models of quasi-crystal structures are based on icosahedral clusters of several layers, on the general lines of those found for the crystalline Mg₃₂(Al,Zn)₄₉ by Bergmann, Waugh and Pauling in 1957 (ref. 4). The problem now is to obtain a detailed proof accounting both for determinate structure and for those statistical departures from it which may account for the present difficulties in allocating atomic positions.

Our understanding of the whole question of 'textures' — the way local order is developed on larger scales — in this context needs re-examination; even the simple case of defining exactly the texture of a drawn metal wire is not clear. Here the structural elements are crystalline grains of certain spread of grain size and orientation. The X-ray diffraction spots are each caused by a number of grains.

As long ago as 1931, Carl Hermann⁵ pointed out that textures could have icosahedral point group symmetry. The geometrical 'Penrose tiling' produces such a texture. In this tiling, the projection of a six-dimensional (hypercubic) lattice into three dimensions gives a tiling of three-dimensional space by two quasi-unit cells, two kinds of rhombohedra, having the same faces but one obtuse and the other

acute⁶. The Penrose tiling is an algorithm for filling space with these two rhombohedral 'quasi-unit cells' such that the resulting array of quasi-lattice points has icosahedral diffraction symmetry if a sufficient volume is considered. It is thus an algorithm for giving a texture with no statistically indeterminate elements.

In orthodox crystallography it is only convenience that leads us to use parallelepipedal unit cells repeating by translation. We could as well use space-filling cells: indeed, this would be more realistic for the hexagonal system, where hexagons correspond better to the physical interactions than the conventional crystallographic parallelepipeds. Correspondingly, in quasi-crystals we could use rhombic triacontahedra, each of which could be made of 10 acute and 10 obtuse rhombohedra, as the physical units, and allow them to overlap in the way prescribed by the Penrose tiling. This is perhaps a more physical way of building in the local icosahedral symmetry.

Unfortunately, the allocation of six-dimensional crystal indices from scattering data involves an ambiguity. Any real number can be expressed (to a prescribed accuracy) as $a + b\tau$, where a and b are integers and τ is irrational, for example the golden number. Thus, as any point in the three-dimensional reciprocal space determined from a diffraction pattern can be given integer indices, two for each dimension, so the allocation of unique indices is not a convincing test for a particular geometrical model. The test of the existence of a particular generation of a quasi-lattice corresponding to a packing of rhombohedra is luckily more stringent and in practice all observed reciprocal lattice points can be attributed to the reciprocal lattice corresponding to a rhombohedral side of length 4.6 Å.

The mathematical theory of how to dispose asymmetrical units in these tiles is not yet properly developed and structure analysis is thus at the pre-space-group stage. Cahn and Gratias⁷ have made a beginning by examining the extinctions produced by projecting centred 6-

dimensional hypercubic lattices. Thus, a potentially complete theory is available for producing a structure which has the required diffraction effects and which has no statistical features at all.

Knowles and Stobbs³ constructed small regions of this kind of structure and calculated diffraction patterns by regarding the structures as large molecules. They find that on the basis of arranging atoms in the two kinds of unit cells no particular structure looks convincing. In agreement with other authors (S. J. Poon, S. Preische & Y. Shen; personal communications) they find that a model with an average atom at the quasi-lattice points gives better agreement than any plausible arrangement of real atoms.

The next question to answer is what kind of disordering is necessary to produce this averaged structure. One possibility is a phase-shift disorder — the staggering of the five lines or columns of atoms in quasi-crystals by half a spacing. High-resolution electron microscopy suggests that the Bragg electron-density waves, which are measured in amplitude and direction (but not in phase) by the sharp diffraction spots, can be phase-modulated (with a range of wavelengths of about 80–100 Å). These dislocations are called phasons. Adjacent grains scatter coherently for the same reflection but with different phases. Anti-phase domains would be an example of a crystal structure broken up by the occurrence of phasons of magnitude π , causing extinctions and variations in spot intensity. A crude summary of the process is the appearance with rapid cooling of many nuclei that are sufficiently mobile to rotate into close angular correspondence with each other over micro-metre ranges. The icosahedral group is optimal as it has the highest order (60).

It looks as if the techniques of X-ray diffraction from a substantial volume may not be sensitive enough to determine both a definite structure and its local disordering and that a model, having passed this hurdle, must also account for electron microscope observations where phase information is also available. Phasons will not be localizable as diffraction methods measure only structure amplitudes and sufficient phasons would perhaps tend to make all reflections much the same intensity, as Knowles and Stobbs observe. □

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Neurobiology

Second messenger dualism in neuromodulation and memory

from Michael Berridge

A recurring theme in neurobiology is that neuromodulation is the key to understanding how the brain works. More often than not such modulation is mediated by intracellular messengers controlling the opening or closing of potassium channels. A classic example is presynaptic facilitation in neurones of the marine invertebrate *Aplysia*, where cyclic AMP reduces the effectiveness of potassium channels, prolonging the depolarizing phase of each action potential and resulting in a greater release of transmitter¹. This cyclic AMP-dependent facilitation can persist for long periods and is therefore a possible mechanism for memory. The article by Higashida and Brown on page 333 of this issue², taken together with several recent studies, provides compelling evidence that the ubiquitous inositol lipid signalling pathway has a key role in neuromodulation. More speculatively, it may extend to the acquisition of long-term memory.

A characteristic feature of this inositol lipid receptor mechanism is that the transduction process has a bifurcation point leading to the generation of two second messengers. A precursor lipid (phosphatidylinositol 4,5-bisphosphate), located within the inner leaflet of the plasma membrane, is cleaved in response to hormones or transmitters to yield the second messenger duet of diacylglycerol and inositol 1,4,5-trisphosphate (Ins(1,4,5)P₃). The latter functions to mobilize calcium³ whereas diacylglycerol activates protein kinase C (ref. 4) giving a highly versatile messenger system that controls an array of physiological processes including contrac-

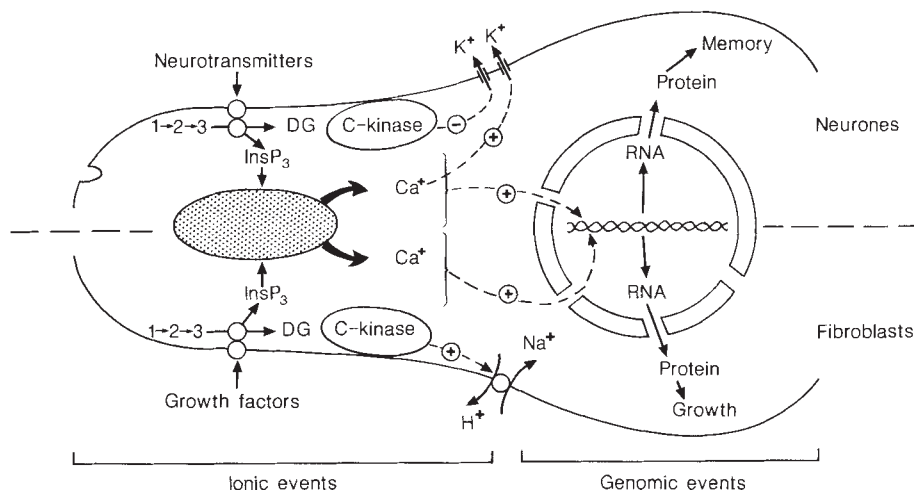
tion, secretion, metabolism, sensory transduction, fertilization and cell growth. The involvement of this system in neural function has been suspected for some time because the brain has a plethora of receptors all linked to this receptor mechanism, but it has proved difficult to find out how Ins(1,4,5)P₃ and diacylglycerol might act in identifiable neurones.

Higashida and Brown² approached this problem by studying a neuronal cell line that responds to the potent pain-producing agent bradykinin with a characteristic early hyperpolarization followed by a longer-lasting depolarization. These two phases of the electrophysiological response are controlled separately by the two limbs of the dual messenger system. Calcium released by Ins(1,4,5)P₃ opens a potassium channel to give the initial hyperpolarization. The subsequent depolarizing phase results from the closure of separate potassium channels (the M-current) controlled by the other limb of the pathway because it can be mimicked by adding agents such as the phorbol esters that activate protein kinase C.

These studies on this neuronal cell line are consistent with observations on nerve cells *in situ* which also suggest that potassium channels are a focal point for the second messenger duet. It has already been suggested⁵ that protein kinase C functions in hippocampal neurones, where phorbol esters greatly potentiate synaptic transmission, either by sensitization of the exocytotic mechanism to the stimulatory action of calcium or by the closure of potassium channels. Evidence

for the latter mechanism already exists: calcium-activated potassium currents responsible for accommodation in hippocampal pyramidal cells are blocked by the activation of protein kinase C (ref. 6). Excitability may be enhanced further by blocking of a voltage-sensitive chloride current by protein kinase C (ref. 7). Closure of potassium channels by protein kinase C could explain the enhanced excitability seen in neurones during the action of substance P (ref. 8) and noradrenalin⁹, both of which are known to stimulate inositol lipid hydrolysis in the brain. A general picture begins to emerge — neuromodulation in mammalian neurones depends on the opening or closing of ion channels (primarily potassium) by the second messenger duet of Ins(1,4,5)P₃ and diacylglycerol. Might these mechanisms underlying neuromodulation contribute to the long-term changes responsible for memory?

One model for memory presented recently by Goelet *et al.*¹⁰ draws an analogy between the mechanisms that control cell growth and the processes responsible for the acquisition of long-term memory. In both cases, early second-messenger events bring about rapid ion fluxes that are causally related to the onset of the genomic events leading to the long-term commitment either to grow or to undergo the stable alteration in neural processing that underlies memory. In the case of cell proliferation, certain growth factors use Ins(1,4,5)P₃ to mobilize calcium and diacylglycerol to activate the Na⁺/H⁺-exchanger that increases intracellular pH (ref. 3). These early second-messenger and associated ionic events, particularly the increase in intracellular calcium, are required and may actually be responsible for the rapid transcription of oncogenes such as *fos* and *myc* that lead to a stable commitment to divide. Goelet *et al.*¹⁰ argue that a similar sequence of events may be responsible for memory. Stimulating neurones at a particular frequency for a certain period of time triggers early ionic events that are followed by the onset of gene transcription and protein synthesis required for the stable alteration in neural circuitry. The crux of the model is that the 'messengers' responsible for the early ionic events are also responsible for



Proposed role of inositol lipid hydrolysis in the control of growth in fibroblasts and the acquisition of memory in neurones. DG, diacylglycerol; 1, phosphatidylinositol; 2, phosphatidylinositol (4)-phosphate; 3, phosphatidylinositol (4,5)-bisphosphate; InsP₃, inositol (1,4,5)-trisphosphate.

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switching on nuclear events.

What these messengers are is still a mystery, but $\text{Ins}(1,4,5)\text{P}_3$ and diacylglycerol must be likely candidates because of their involvement in neuromodulation. A role for calcium has already been implicated in PC12 cells where there is a rapid increase in *c-fos* transcription following the addition of agents that open voltage-dependent calcium channels¹¹. Long-term potentiation of synaptic transmission between neurones in the hippocampus has been used as an experimental model for memory. Because both calcium and protein kinase C have been implicated in the acquisition of long-term potentiation there already is some experimental evidence for the involvement of such messen-

ger systems in memory¹². The photoreceptors of *Hermisenda* provide another experimental model for memory where the closure of potassium channels by protein kinase C is an essential component of the plastic change that occurs during associative learning¹³. The virtuosity of this dual signalling system is truly remarkable: not only does it control the second-to-second activity of most cells, but it can apparently extend its performance to controlling the long-term plastic changes that underly cell growth and the acquisition of memory. $\square$

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Particle physics

Glueball sightings premature

from Frank Close

Has a glueball at last been positively identified? Aihara *et al.* (*Phys. Rev. Lett.* **57**, 51; 1986) seem to think so; their measurement of the lack of electromagnetic coupling of the ι particle leads them to conclude that the ι is the long-sought bundle of gluons. Certainly the ι has been most people's favourite candidate for some years but a definitive test has been awaited. The present claim comes from data which are some 40 per cent better than an earlier experiment by the 'TASSO' collaboration in Hamburg (Althoff *et al.* *Z. Phys.* **C29**, 189; 1985), but this group did not risk such a positive claim, and the evidence is perhaps not as clear-cut as the new data of Aihara *et al.* seem to imply.

Atomic nuclei are made of protons and neutrons which are in turn clusters of quarks. Quarks possess an unusual type of charge called colour which operates in some ways like electrical charge. The relativistic quantum field theory of the colour-induced forces is called quantum chromodynamics (QCD) by analogy with the mathematically similar theory of quantum electrodynamics (QED). The photons of QED transmit electromagnetic forces; similarly gluons transmit the chromodynamic forces in QCD.

These similarities induce theorists to seek to build a unified theory of these forces. But first, is QCD correct? If it is, then in addition to ordinary matter there should exist glueballs — matter made of glue not of quarks. There are no balls of light in QED because photons are electrically neutral and do not mutually attract. However, the gluons of QCD carry colour charges and so mutually should attract by the same forces that cluster the quarks into conventional nuclear particles.

Following the discovery of the ψ (psi)

particle, theorists in the late 1970s realized that QCD implied that radiative decays of the ψ should be a good source of glue matter. Prominent in the decay particles was a hitherto unknown state — the ι (ι), a *prima facie* candidate for resonating glue. It has a mass of 1.4 GeV (about 50 per cent more than a proton) in line with theorists' expectations for glueball masses.

Encouraging as this is, there are some features of its decays which do not fit well with the expectations for a glueball. There is also an unfortunate coincidence that an excited state of the η (η) particle, a quark state, is expected to occur in this vicinity with the same quantum numbers of spin and parity (behaviour of the wavefunction under mirror reflection) as the ι . So the question is: is the ι a gluonic state or an excited η or a hybrid of quarks and glue?

To help answer this, difficult studies of the electromagnetic interactions of the ι have been made. Glue is electrically neutral and so glueballs should have rather feeble couplings to photons. In particular the ι should have a much-suppressed decay into two photons, a mode that is allowed by quantum mechanics.

In 1985 the TASSO collaboration referred to above showed that this mode is somewhat smaller than expected for a quark state at this mass. The experiment of Aihara *et al.* improved this by nearly a factor of two, which although suggestive is hardly conclusive given the various sources of error. There are two problems (Aihara and colleagues comment only on one) which call for more work before their claim is established.

During the past three years there have been reports that ι has a prominent decay into photon and ρ (γ - ρ). This is an

electromagnetic coupling, and is so large that theorists began to suspect that there is significant quark content in ι : it is either an excited quark state or a hybrid. Independent of the interpretation there is a conundrum: the ρ has the same quantum numbers as the photon and so can turn into a photon, this feeding the decay chain ι into two photons. The prominent rate into γ - ρ and this piece of quantum mechanics implies that the decay of two photons should be at least five times larger than the experimental upper limit! This discrepancy must be explained before conclusions are drawn.

One possibility is that there are two states in the 1.4 GeV region of mass: the ι and another. Supporting this is the fact that the mass and width of the signal in the γ - ρ channel seems not to be consistent with the properties of ι determined from other experiments. The true ι might have small couplings both to γ - ρ and two photons; a canonical glueball. The γ - ρ signal might herald the excited quark state expected in this mass region; if so then there should be interesting interference phenomena that will need analysis.

However, there is a less exciting possibility that has already been discussed in a recent Toronto University preprint by T. Barnes. If the ι is an excited quark state (η^*) then in addition to its copious γ - ρ decay it will also have a powerful coupling to γ - ρ^* (an excited ρ analogous to the excited η^*). This latter decay channel is closed by energy conservation but it can contribute destructively to the amplitude for ι decaying into two photons. The apparent anomaly between the small γ - γ and large γ - ρ is then due to neglect of the (even larger) destructive γ - ρ^* coupling. If this is the explanation in decays of the ρ^* : $\rho^* \rightarrow \iota + \gamma$; or in electron-positron annihilation: $e^+e^- \rightarrow \gamma \rightarrow \rho^* + \iota$.

Until the opening of the large electron-positron collider (LEP) in Geneva or the Stanford Linear Collider (SLC) and the hoped-for discovery of bound states of top quarks (whose decays should be a copious source of glueballs), it is most promising to continue studying ψ decays, or low-energy annihilation of protons and anti-protons (as at LEAR in Geneva) for sightings of glue. If there are several spinless particles with negative parity (pseudoscalars) in the 1 to 2 GeV mass range (as data suggest), then quarks will be unable to accommodate them all. Gluonic degrees of freedom will need to be invoked. Careful examination of pseudoscalars, and clarification of the electromagnetic coupling of ι , are therefore probably the best current strategies. $\square$

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Magnetic reversals

From the core or the skies?

from J. A. Jacobs

THERE has been a flurry of papers in the past two years on reversals of the Earth's magnetic field and their possible connection with extraterrestrial catastrophic events. What has sparked this sudden interest is the reported periodicity of approximately 30 million years (Myr) in the frequency of reversals, comet/asteroid impacts on the Earth and mass extinctions.

There are many analyses of the frequency distribution of reversals of the Earth's magnetic field using statistical approaches (for example, refs 1–3) and it is not surprising that some of them disagree. Several harmonics, including one at 32–34 Myr¹ and one at 15 Myr¹² were reported. Later, Raup³ claimed a 30-Myr periodicity in phase with a 15-Myr signal. The reality of a 30-Myr signal was questioned by Lutz⁴ who showed that it is sensitive to the length of the time series, so that when the record is truncated by progressively omitting the most recent events, the spectrum changes. Thus, the 30-Myr peak in the spectrum seems to be an artefact of the record length, and Raup, in a *News and Views* article⁵, concurred. But in the most recent analysis, Stothers⁶ holds that a statistically significant periodicity of about 30 Myr does exist. Pal and Creer⁷, although not suggesting a 30-Myr periodicity, claim that the palaeomagnetic record shows 'spurts' in the frequency of reversals separated by approximately 30-Myr intervals.

It is not difficult to imagine that the impact of a large body on the Earth's surface could lead to mass extinctions. Many have proposed different scenarios for the effects of such an impact, although there is consensus that it would have almost immediate consequences with the loss of many species. There are, of course, other possible causes of extinctions, and nobody proposes that they were all caused by the impacts of large bodies. The most publicized case for an extraterrestrial cause of mass extinctions occurred about 65 Myr ago at the Cretaceous–Tertiary boundary. Deep-sea limestones show marked increases in the concentration of iridium above the background level at precisely the time of the Cretaceous–Tertiary extinctions, and Alvarez *et al.*⁸ claim that this iridium is of extraterrestrial origin. One of the best examples can be seen in the pelagic limestones at Gubbio in the Umbrian Apennines of Italy, which also permit detailed palaeomagnetic studies. The planktonic foraminiferal change, and hence the Cretaceous–Tertiary boundary, in this region is not coincident with any particular polarity change⁹.

Quite apart from the difficulty of the

absolute dating of stratigraphic boundaries, a fundamental problem arises in the culling of the database and in deciding what is mass extinction and what background extinction. Hoffman¹⁰ showed that using different (but plausible) geological timescales and other (acceptable) definitions of mass extinctions nullifies the evidence for any periodicity. Several authors took issue with Hoffman's criticisms in a *Nature* Matters Arising exchange¹¹. But Hoffman¹¹ answers their objections and maintains that, "although there may be a periodicity in late Permian to Quaternary extinctions, the evidence is as yet insufficient".

Despite these uncertainties, there have been several attempts to find an astronomical explanation for a correlation between mass extinctions and extraterrestrial events. One attributes the correlation to oscillations of the Sun perpendicular to the galactic plane (estimated to be 33 ± 3 Myr)¹². Biological crises could arise as a result of collisions with interstellar clouds of gas and dust that are concentrated towards the galactic plane; a nearby interstellar cloud would perturb the family of comets in the Solar System, leading to an increased flux of bodies impacting the Earth¹².

The 'periodicity' in extinctions could also be controlled by an unseen companion of the Sun (Nemesis) in a highly eccentric orbit that periodically brings it into the dense inner region of the Oort cloud of comets, perturbing the orbits of many of them and initiating a comet shower resulting in several terrestrial impacts¹³. Among various objections to this hypothesis, Hut¹⁴ estimates that irregularities in the period of revolution of the supposed 'double-star' over the past 250 Myr would be at least 10 per cent, and more likely 20 per cent. One would thus not hope for or expect perfect correlation between crater impacts and mass extinctions. Taken together with Hoffman's¹¹ criticisms of periodicity in the timescale of mass extinctions, correlation between periodic catastrophic extraterrestrial events and mass extinctions seems very unlikely. This does not preclude individual occurrences such as the large body impacting the Earth at the Cretaceous–Tertiary extinction.

What effect would a large impact on the Earth's surface have on its magnetic field? It is generally believed that the magnetic field results from dynamo action in the Earth's fluid outer core. But there is no consensus on what drives motions in the core. The most plausible view now seems to be 'compositionally' driven convection

— freezing of material at the inner-core boundary separating a heavy fraction (mainly iron), leaving behind a lighter liquid fraction in the outer core. If events at the surface of the Earth were to lead to changes in core pressure, this would affect the role of freezing of the liquid core and modify the power supply to the dynamo. For a body of mass 10^{15} kg impacting the Earth, the change in power supply is estimated to be only 0.2 per cent¹⁵. There may also be a deep-mantle poroelastic shell within the Earth capable of assuming two states, inflation or deflation¹⁶. As the shell inflates or deflates, poroelastic stresses are redistributed in the core, modulating its solidification and affecting the polarity of the magnetic field. In this model¹⁶, changes in pressure are again invoked to change the polarity, but pressure changes are caused by events internal to the Earth and not extraterrestrially induced.

The cause of reversals may be the result of fluctuations in the net helicity of the core (a measure of the correlation between turbulent velocity and vorticity) in response to fluctuations in the level of turbulence produced by two competing energy sources: thermal convection and growth of the inner core¹⁷. Helicity generated by heat loss at the mantle–core boundary has the opposite sign to that generated by energy release at the inner core boundary. The possible effect of pressure changes at the inner-core boundary caused by the impact of large bodies at the Earth's surface has been shown to be negligible — but what about changes in thermal conditions at the mantle–core boundary? It is known that the bottom 200 km of the mantle exhibits anomalous seismic properties: the basic question is whether this boundary layer is compositional/thermal. This question is still controversial. A thermal boundary



100 years ago

THE RECENT EARTHQUAKE IN GREECE

On the 27th inst., at 11.30 p.m., at a distance of 50 miles W. $\frac{1}{2}$ S. from Cape Matapan, I felt, all of a sudden, a very strong shock, which made the ship tremble, especially the engines, for the space of about 11 seconds. At midnight I observed on our right something like a mass of thick black smoke, which, like a cone, was rising up perpendicularly from the horizon, and at intervals changing into a reddish colour. At 10 a.m. the mate, who was on watch on the bridge, reported to me that he had observed in the sea several stripes of a dark yellowish colour about one quarter of a mile long.

CAPT. L. AQUILINA

Nature 34, 497; September 23, 1886.

layer seems to be necessary to produce a significant heat flux from the core demanded by any dynamo mechanism, but an additional composition change cannot be excluded. A large impact at the Earth's surface could not affect the composition of the layer and it is difficult to see how it could substantially affect the thermal regime.

In all physically realistic situations the addition of some noise process (random or otherwise) must intrude to influence the evolution of the system. Work in my laboratory¹⁸ shows that the addition of three types of stochastic processes (gaussian, flicker and brown noise) enriches the field evolution and can predict accurately palaeomagnetic reversal behaviour. Irregularities either in material properties, physical or chemical processes or in fluid dynamical turbulence could provide an adequate source of noise. It seems much more probable that this external stimula-

tion by a random component is internal to the core and is not caused by an extraterrestrial event. □

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Geophysics

Mantle mixing still a mystery

from Dan McKenzie

PRINCIPALLY because of the work of isotope geochemists, it is now clear that the Earth's mantle contains certain regions of significantly different Sm/Nd and Rb/Sr ratios, which have probably existed for at least 1,000 million years. All oceanic islands are produced from such regions. If their geometry and size distribution were known they could be used as strong constraints on the history of mantle circulation. The difficulty lies in deciphering the geological observations, a problem addressed by two papers, one recently published in *Nature*¹ and the other on page 317 of this issue².

The most impressive feature of volcanic rocks dredged from the ocean floor is not their heterogeneity but their homogeneity: they are almost all classified by petrologists as one particular type of basalt. This uniformity extends to the isotope ratios ⁸⁷Sr/⁸⁶Sr and ¹⁴³Nd/¹⁴⁴Nd, which are measures of the average Rb/Sr and Sm/Nd ratios over periods comparable to the age of the Earth. The most obvious way in which such a homogeneous source could be produced is by mixing in the dust cloud from which the Earth formed. But all geochemists who have worked on the problem agree that the homogeneity did not originate in this way, because the source shows clear signs of depletion of Nd and Sr at some later time in its evolution. This depletion event is generally believed to be the formation of the continents, whose average age is about 2,000 million years. The standard type of geochemical modelling, with fluxes between uniform reservoirs, accounts for the

observations³⁻⁵, but provides no clue as to how the homogenization occurs.

Because the depletion is widely believed¹ to occur at island arcs, and therefore to be very localized, the homogenization must be both fast and efficient. Recent studies of two-dimensional convection, reviewed by Allegre and Turcotte¹, show that vigorous two-dimensional time-dependent convection can (just) achieve the necessary homogenization. However, if the Rayleigh number of the convection is too small, time-dependent flow does not occur and the stirring occurs only within one convective cell. Efficient stirring occurs over larger regions only when the flow is time-dependent. Then the particle paths are not stream lines, so the description of such stirring as stream-line mixing¹ may be misleading. Recent work on lead isotopes⁶ suggests that the stirring time may be as short as 500 million years, considerably less than the estimates from two-dimensional convection. Because any cook knows how much more effective three-dimensional stirring is than two-dimensional, such a difference is not surprising as mantle convection is certainly three-dimensional.

Vigorous stirring will not by itself produce a homogenous mantle, but one in which inhomogeneities are reduced to thinner and thinner streaks. Only when their size is less than a few tens of centimetres will solid-state diffusion produce true mixing. The question which has dominated discussions of mantle heterogeneity for the past 10 years is whether it

is possible to extract the necessary volumes of basalt to make oceanic islands from thin streaks of enriched mantle without involving the thicker depleted streaks in between? That this argument is not settled is clear from the two recent *Nature* papers^{1,2}, which take opposite sides on this question. In my view the only way of doing so is through understanding the physical processes involved in the generation of melt and its movement to the surface. Although such studies are progressing rapidly, they are not yet able to resolve the question. Until it is resolved it is unclear whether melt extraction acts to increase¹ or to decrease² the magnitude of the isotope anomaly on some length scale. Mathematically, the mapping of the mantle heterogeneities into surface anomalies by melting is non-linear and the functional form is unknown.

Why not avoid all these problems by studying pieces of the mantle which are now at the surface, as Allegre and Turcotte propose? The difficulty with this approach is that nasty things happen to the mantle on its way to the surface. The mantle starts its journey at a temperature sufficient to produce melting if it were at atmospheric pressure, and is also likely to be strongly deformed on its way up. My first attempt⁷ to study mantle structures directly was motivated by the crystal geometries within pieces of mantle brought up with diamonds. It later became clear that these fabrics would last less than half an hour under mantle conditions. The observed structures were related to the extraction event, not to the much slower mantle circulation. The most obvious method to demonstrate that the pyroxene bands in peridotite¹ are long-lived features, and are not formed by melting and deformation during uplift and emplacement into the crust, is to measure the isotope ratios in the different layers. If the observed ratios correlate with the banding, some estimate of its age could be made. Unfortunately, no such correlation is observed¹, probably because of the movement of small quantities of volatile-rich melt into the peridotite which transported Sr and Nd.

It is disappointing that all attempts to determine the geometry of mantle heterogeneities have been equally inconclusive. As is so often the case, the problem is important but appears to be at present unanswerable. □

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Marine biology

Stunning whales and deaf squids

from Michael Alan Taylor

SOME odontocete cetaceans (toothed whales and dolphins) stun prey with intense bursts of sound from their sound echolocation, or sonar, apparatus¹. Recently, it has been suggested² that the apparent deafness of coleoid cephalopods (squids, cuttlefishes and octopuses) is an adaptation to resist these attacks. So much for the weapon — but what of the tactics?

It may well be that deafness is a handicap against these attacks. Before the

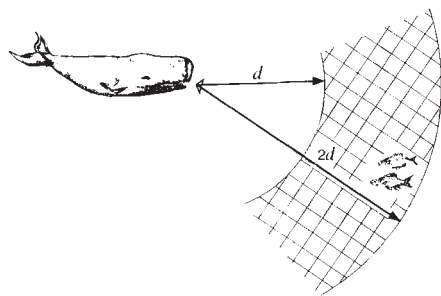


Fig. 1 Echolocating whale and prey. At the maximum detection distance d , the sound has to travel $2d$. Prey are safe in the shaded area if predator and prey have equally sensitive ears.

whale can launch a stunning attack, it must first locate the prey by emitting bursts of sound energy and listening for reflected echoes. The intensity of sound is inversely proportional to the distance travelled, so it declines both on the way to the target and, as the echo, on the return to the whale. At maximum detection range, when the whale can only just hear the echo, the sound it emits will be equally audible at twice the radius from predator to prey, as the sound has continued past the prey. Moreover, this assumes that all the sound energy hitting the prey is reflected back to the whale. In practice, some will be reflected in other directions, some will be absorbed and some scattered around the prey, especially if the prey is of the same order of size as the wavelength of the sound³. So, if the whale and prey have equally sensitive ears, the prey can hear the searching whale at least twice from as far away as the whale can detect it (Fig. 1).

The value of receiving early warning of a searching whale allows the prey a margin of safety in time and distance. It is true, as suggested², that simple on-off receivers only serve to distract the prey if they do not specify the direction, range and relative hazard posed by a threat: but once such information is used the receivers become vastly more useful. Qualitative information can in principle be obtained from, for example, the click-frequency rate of whale sonar, indicating whether the animal is merely searching or has

actually detected prey and is intercepting.

So why should cephalopods be deaf, if by hearing they can avoid sonic stunning attacks? The fossil record suggests that the deafness of cephalopods is not a response to whales. All modern coleoids are deaf and the most parsimonious hypothesis is that their latest common ancestor was deaf. This common ancestor must have existed in the Lower Jurassic or before, because the coleoids had by then radiated into their present groups, about a hundred million years before the first known cetacean, *Pakicetus*, from the Eocene^{4,5}. Moreover, none of the predatory marine reptiles of the Jurassic and Cretaceous possessed the cranial and auditory adaptations needed to emit and receive echolocation sound — and still less to carry out stunning attacks — comparable with those of odontocete whales. So, even if the deafness of cephalopods is useful in resisting sonic stunning, it cannot be a primary adaptation but is, rather, a secondary use.

Moynihan² assumes that the deafness of cephalopods is adaptive, but others have argued^{6,7} that the anatomy of any animal is determined only partly by the ideal structure implied by the functional demands on it; other limits are imposed by its evolutionary history, constituent materials and developmental programme. For example, do the surges of internal and external pressure caused by jet propulsion interfere with hearing?

One reason why prey animals congregate in schools may be to confuse the predator trying to intercept one individual animal⁸. Two further functional reasons for sonic stunning are first, that it acts as a 'shotgun' against multiple targets hard to track on sonar; and second, by using the same medium of energy transmission as the tracking system, it avoids any errors caused by trying to translate a sonar 'fix' to a visual interception.

Do fishes and squids possess countermeasures to whale sonar? The obvious defence of evasion on hearing a whale cannot apply to cephalopods because they are deaf, and it is not clear whether fishes can hear and recognize whales' echoloca-

tive sounds, partly because little is known about fish hearing⁹. But the prey can do just as well by not being detected in the first place: by hiding near some environmental feature that reflects sound strongly, or by evolving a body shape and composition that reflects sound less well. The air-filled buoyancy chambers of early cephalopods reflect sound strongly, as do the swim bladders of fish. Squid and scombrid fishes such as mackerel and tuna that swim in open water have lost their flotation tanks in favour of hydrodynamic lift. The cuttlefishes have retained their air-filled shells for flotation, but because they tend to swim or hover over the bottom¹⁰ they must be hard to detect on sonar.

So the squids and scombrids appear to show excellent examples of stealth design. But a more critical look at their evolutionary history shows that squids existed in their present form long before the whales appeared in the Eocene^{5,11}, whereas the scombrids appeared in the Eocene, possibly before sonar-equipped modern whales (Fig. 2). In fact the reliance of these animals on hydrodynamic lift, rather than air-filled chambers, can be explained on energetic grounds¹²: hydrodynamic control of buoyancy produces less drag than gas-filled tanks in these fast, constantly swimming animals. The buoyancy chambers of cuttlefish are more efficient for slow swimming and allow the animals to halt and hover over the bottom while searching for food. So, although the loss of air-

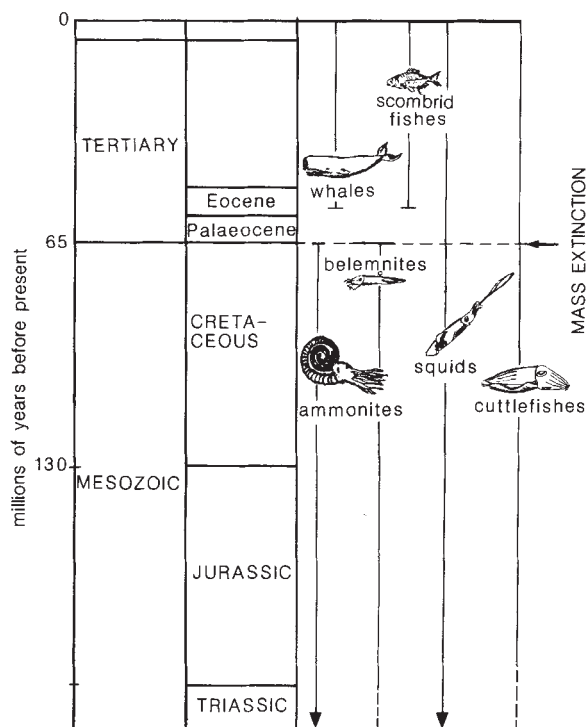


Fig. 2 Evolution of whales, fishes and cephalopods, showing how the loss of an air-filled buoyancy chamber (presumed adaptation against whale sonar) precedes (squids) or does not appear long after (scombrids) the first whales. Animals with buoyancy chambers became extinct because of the Cretaceous-Tertiary mass extinction and not predation.

filled chambers is an important adaptation against whale sonar, it is primarily for locomotor efficiency and only secondarily for anti-sonar defence.

Other obvious defences include jamming, decoying and mimicry. There seems to be little scope for jamming or decoying given the vastly greater sound-producing powers of whales over fishes and squids, and it is hard to imagine how an animal can produce an expendable decoy that resembles it sufficiently on sonar. Perhaps the most promising is mimicry of a distasteful species. Do toxic fish emit ultrasonic cries when they hear whales and do edible fishes make similar calls?

If fishes and squids have sonar counter-measures, have whales kept ahead in the evolutionary arms race by using sonar more efficiently, or even by using it only intermittently as part of a set of techniques, including passive listening for the calls and swimming noises of prey? Some species of bats often use passive listening and vision as well as, or instead of, active sonar¹³, especially when hunting prey which can hear their sonar emissions, and it has already been suggested¹⁴ that sperm

whales lie still at enormous depths, listening for the swimming of giant squids. Another reason for the quietness of whales suggested by Norris and Möhl¹ is to avoid alerting other whales that might want to share the food, or even attack the original whale. □

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Plant molecular biology

Viral RNA in mitochondria?

from David M. Lonsdale

MEMBERS of the largest group of plant viruses contain single-stranded RNA in the '+' or messenger RNA sense. Not all plant viruses induce disease symptoms: the cryptic or temperate viruses, for example, are asymptomatic. Most of these viruses have segmented double-stranded (ds) RNA genomes and virus-like particles can be detected in healthy plants at low levels. In this respect they are similar to dsRNA killer systems in fungi. The presence of dsRNA in healthy plants is often assumed, therefore, to be indicative of a cryptic virus infection¹. However, many apparently healthy plants contain dsRNAs where no inclusion bodies or virus-like particles have been detected². In a recent report³, such cytoplasmically located self-replicating RNAs have once again been highlighted in plants and in particular within the mitochondria of the S cytoplasm of maize.

Cytoplasmically inherited male sterility (CMS), an inability to produce or release functional pollen, results from a mitochondrial-nuclear dysfunction that is common to many higher plant species. In only a few instances, however, have apparently self-replicating dsRNAs been identified in CMS cytoplasm and their role as causative agents of the CMS phenotype has never been established.

Maize has four different cytoplasm types, one (N) is fertile in all nuclear back-

grounds, the remaining three (C, S and T) produce male-sterile plants with certain nuclear genotypes. With one exception, unusual RNAs are limited to the S-cytoplasm group. In the lines W182BN and 2132, two abundant dsRNA species of approximately 2,900 (LBN1) and 900 (LBN2) base pairs were identified by Sisco and co-workers^{4,5}. Analysis of other S-cytoplasm lines, particularly CMS-S(B73 × Mo17) and the two fertile lines R369 (a spontaneous fertile revertant of an S-cytoplasm inbred); and RU (a South American race of maize related to fertile N-cytoplasm types) has revealed two abundant single-stranded RNA species designated S/RU-RNA-a and S/RU-RNA-b (ref. 6).

Hybridization and heat-denaturation experiments demonstrate quite clearly that S/RU-RNA-b hybridizes to LBN2 but not to itself. Following a 60°C formamide denaturation, most LBN2 comigrates with S/RU-RNA-b. The implication of these results is that LBN2 is the replicative double-stranded form of S/RU-RNA-b. It is probable that a similar relationship exists between LBN1 and S/RU-RNA-a (ref. 4). There is evidence to suggest that these RNAs, LBN1 (S/RU-RNA-a) and LBN2 (S/RU-RNA-b), are related, in contrast Schuster and co-workers⁶ have demonstrated that these RNAs are unrelated to other mitochond-

rial DNA plasmids and to the main mitochondrial genome.

The recent paper by Finnegan and Brown³ confirms the presence of both single- and double-stranded RNAs in the S cytoplasm of maize and establishes that they exist in the mitochondrion and not as ribonucleoprotein particles associated with the mitochondrial fraction. By isolating mitochondria and incubating them in medium that supports RNA synthesis in the presence of actinomycin-D, two major labelled products, the dsRNAs LBN1 and LBN2, can be identified. A low level of incorporation of label is also found in the single-stranded forms (S/RU-RNA-a and b) suggesting that these arise from replication or transcription of the dsRNAs. The structure of these RNAs is unknown — the denaturation studies provide more questions than answers⁶.

The labelling of both the double- and single-stranded RNAs in the presence of actinomycin-D is characteristic of RNA synthesis by plant RNA-dependent RNA polymerase, an enzyme of both healthy and RNA virus-infected plants⁷. In the S-cytoplasm group of maize no inclusion bodies or virus-like particles have been observed, although the nuclear genotype does influence strongly the relative abundance of these mitochondrial RNAs⁵.

The available evidence demonstrates that these RNAs are not causally linked to the S male-sterile phenotype in maize as they are present in at least two fertile cytoplasm, nor are they associated with the presence of specific mitochondrial plasmids^{5,6}.

Because they are not related to other mitochondrial sequences it is probable that these intramitochondrial RNA replicons have an exogenous origin. Many plant viral infections are associated with the organelles, therefore these RNAs could be relics of RNA virus infections or an abortive infection where the whole or part of the viral RNA genome has been sequestered by the mitochondrion and is now being maintained by the plant RNA-dependant RNA polymerase. These RNA replicons constitute a further unusual feature of plant mitochondria in addition to the plethora of circular and linear DNA plasmids which exist and which are unrelated to the mitochondrial genome. □

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Mystery object amid the chromosomes

SIR—The very tiny object shown below, much like a fragmented crossword in appearance, was recently found in one of our routine chromosome preparations for prenatal diagnosis following amniocentesis. But what is it?

Is it a man-made device? Packing text as binary coded information on the miniature scale (the scale bar is 10 μm) would seem advantageous. Or is it a naturally occurring substance? None of the possibilities we have been able to think of would



seem to be appropriate for amniotic fluid, so if anybody is able to suggest an answer to this mystery we would like to have it. We are as intrigued as we are ignorant.

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Prospects for rabies control by vaccination

SIR—The successful vaccination of foxes against rabies using a recombinant vaccinia virus expressing rabies G glycoprotein¹ is an exciting advance and one can only look forward to field trials with great anticipation. But R.M. Anderson's News and Views summary² of this work contained some omissions and errors.

Anderson states: "... the instability of the attenuated strains and the possibility of reversion to full virulence have prevented their use in natural habitats". One awaits data on the stability of the vaccinia recombinant but for the past several years, thousands of doses of live attenuated rabies vaccines have been released in Switzerland, West Germany and Ontario, Canada. Not only have these releases apparently been effective in preventing the spread (Switzerland) or reducing the incidence (Germany) of rabies, but the

trials have been conducted without any evidence of reversion to virulence. Given the hurdles that face us in obtaining approval for field trials of any recombinant DNA products and the continuing severe global threat of rabies, use of attenuated live-virus vaccines is likely to continue for many years.

Anderson's analysis of the proportion of a wild fox population that must be vaccinated in order to interrupt or prevent a rabies epidemic is based on a model; clearly, his predictions are only as good as the data entering the model and its structure. None of the several rabies models has been adequately field tested. When they are, we may get indications that suppression (if not eradication) of rabies may be obtainable with much lower proportions of immune individuals than is supposed.

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Body temperature and the specific heat of water

SIR—The majority of homoiothermic animals maintain their body temperatures, during non-hibernation, within a few degrees of 36°C. Various explanations for this have been advanced, most centering on the temperature-dependent change in rate constants of enzyme-mediated chemical reactions. These explanations appear to beg the question, why 36°C? Why not 25°C or 48°C? Is it possible that the enzyme systems of cells evolved as a result of being encouraged to work at 36°C rather than the reverse? If so, the 36°C setpoint assumes some importance and a reason for its occurrence in 'warm blooded' organisms must be sought.

The reason seems to be found in the table recording the specific heat of water of Kaye and Laby's *Tables of Physical and Chemical Constants* (page 55, 14th edn, Longman, London, 1973). As usual the key to much of life's mystery lies in the extraordinary behaviour of water. As plotted in the figure, the relationship between the specific heat of water and temperature reveals that at 35°C the specific heat of water is at its minimum value of 4.1779 J g⁻¹ °C⁻¹.

In the case of living organisms the significance of this strange behaviour of water is immediately obvious. An organism functioning at this temperature will find it necessary to generate or dissipate the minimum amount of heat energy in order to maintain its temperature constant. From the point of view of the organism's energy economy this temperature is clearly the most efficient at which to function. It seems likely that since the environmental temperatures on Earth are,

with few exceptions, lower than 35°C, organisms which have been able to set their working temperatures at a point just

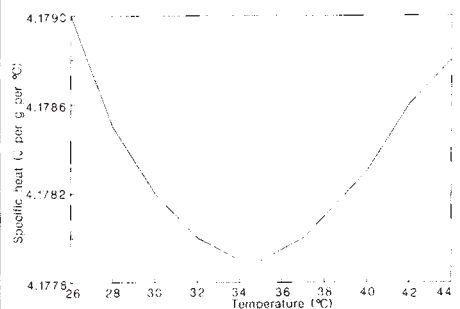


Fig. 1 Specific heat of water as a function of temperature.

slightly above the temperature at which the specific heat of water is at its minimum value have thrived. Some of your readers may care to elaborate further on the implications of this observation.

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FRP is follicle regulatory protein

SIR—Confusion may be caused by the use of FRP as the acronym for the FSH releasing protein in follicular fluid described by Vale *et al.* (*Nature* **321**, 776–779; 1986). Since first reporting the presence of a follicle regulatory protein in the ovary in 1982¹ we have referred to it as FRP in numerous publications, for example ref. 2. FRP, which has a role in both ovarian and testicular physiology as a paracrine and autocrine modulator of gonadal response to gonadotropins, is biochemically similar to Vale's protein and the two may even eventually turn out to be the same. However until and unless they do, one FRP is surely enough.

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Scientific Correspondence

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High-latitude salinity effects and interhemispheric thermohaline circulations

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A general circulation model for the ocean is used to investigate the interaction between the global-scale thermohaline circulation and the salinity distribution. It is shown that an equatorially asymmetric circulation can be maintained even under equatorially symmetric basin geometry and surface forcing. Multiple equilibrium solutions are obtained for the same forcing by perturbing the high-latitude salinity field in an otherwise equatorially symmetric initial condition. The timescale of the transition from the symmetric circulation to an asymmetric circulation depends critically on the sign of the initial salinity perturbation.

THE thermohaline circulation is that part of the total ocean circulation which is driven by fluxes of heat and fresh water through the sea surface, and is often pictured as an overturning of the oceans in the meridional-vertical plane. In contrast to the quasi-horizontal wind-driven gyres, which are confined to limited ranges of latitude, the thermohaline-driven overturning cells are global in scale, and there is a high degree of asymmetry in the sense and intensity of the circulation between basins. The interhemispheric and inter-basin water mass exchanges associated with the thermohaline circulation have an important influence on the distribution of properties in the deep ocean¹, and on global-scale heat and fresh-water transports². Analyses of palaeoclimatic data^{3,4} suggest that the strength and pattern of the thermohaline circulation have changed significantly between glacial and interglacial periods, and several authors⁴⁻⁶ have proposed (largely qualitative) theories for glacial/interglacial climate variability in which the thermohaline circulation plays a central role. A common element of each of these theories is the interaction between the thermohaline circulation and the salinity distribution. The study described here attempts to model these interactions in a quantitative, dynamical framework, using a three-dimensional general circulation model of the ocean.

Role of salinity

Our present understanding of ocean dynamics is insufficient to explain the differences in the sense or magnitude of the thermohaline circulation between basins, or how they might change with time. Several studies^{7,8}, however, have pointed to the importance of the high-latitude salinity field in controlling the location of deep water formation and in driving interhemispheric circulations. Deep water formation occurs in only a few localized regions, primarily in the high-latitude North Atlantic and around the Antarctic continent⁹. There is a conspicuous lack of deep water formation in the North Pacific. It has long been recognized that this is due to the low surface salinities in the sub-polar North Pacific, which prevent surface water cooled even to the freezing point from overturning with the more saline deep waters¹⁰. A related, and in fact inseparable problem is to explain what maintains the salinity difference between ocean basins⁷.

Relatively few ocean modelling studies have included salinity as a prognostic variable. There are a number of differences between the effects of temperature and salinity, however, which are important in the context of the large-scale thermohaline circulation. First, the equation of state of sea water is nonlinear, so that the relative importance of temperature and salinity variations in the buoyancy field depend on the temperature and salinity themselves. Temperature variations dominate the

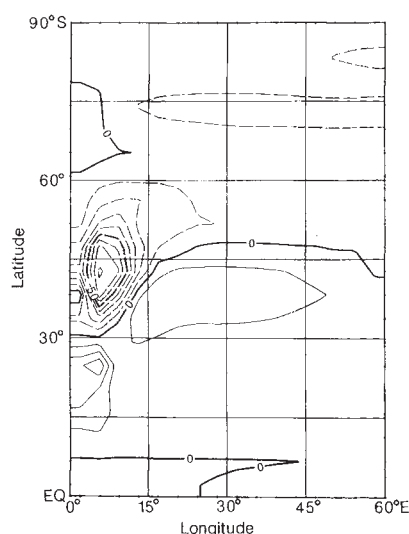


Fig. 1 Virtual surface salt flux distribution used for all experiments, $\text{mg m}^{-2} \text{s}^{-1}$.

buoyancy distribution at high temperatures and salinity variations dominate at low temperatures (and hence at high latitudes, where deep water forms). Secondly, there is an important difference between the nature of their respective surface forcings. There is a strong feedback between sea surface temperature (SST) and its atmospheric forcing through the temperature dependence of latent and sensible heat fluxes and long-wave radiation. Surface salinity, on the other hand, has negligible direct effects on evaporation and precipitation, which provide its surface forcing. Many of the ocean modelling studies which did include salinity neglected to take this latter difference into account. In addition, it is common in ocean^{11,12} and climate¹³ modelling to use highly simplified continent-ocean geometries and to impose a symmetry condition at the Equator, to save computational resources. An equatorial symmetry condition, however, does not allow interhemispheric thermohaline circulations, which are a real and important part of the climate system.

The model and surface forcing

The general circulation model is based on a finite difference formulation of the primitive equations of motion^{14,15}. It includes both temperature and salinity as prognostic state variables, and uses a polynomial approximation of the equation of state¹⁶. The basin geometry is a 60°-wide sector of the sphere extending from pole to pole, with a flat bottom at 5 km depth. The horizontal resolution is 3.75° longitude $\times$ 4.5° latitude, with 12 levels in the

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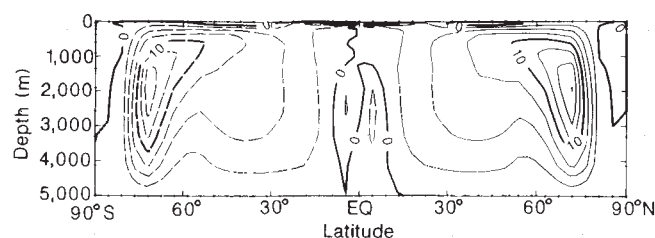


Fig. 2 Stream-function for the zonally integrated meridional overturning circulation for experiment 1 at the end of the integration. Positive values indicate a clockwise circulation; contour interval, $2.5 \times 10^6 \text{ m}^3 \text{ s}^{-1}$.

vertical dimension. The choice of model parameters is such as to suppress any time variability arising from hydrodynamic instabilities. Sea ice is not taken into account. A complete description of the model physics and numerical implementation are provided elsewhere^{11,15}.

A wind stress is applied, which approximates the observed zonal mean stress. The surface heat flux is parameterized by a newtonian cooling law¹⁷, in which the flux is linearly proportional to the difference between the predicted SST and a prescribed zonal mean equilibrium temperature (taken as the observed¹⁸ zonal mean SST, averaged over all oceans and both hemispheres). This type of boundary condition can also be thought of as restoring the model SST towards the prescribed distribution on a timescale taken here as 25 days. The salinity surface boundary condition is a fixed virtual salt flux (to represent evaporation, precipitation and runoff). Thus, as discussed above, whereas there is a feedback between the surface heat flux and surface temperature, the surface salt flux is independent of surface salinity. All boundary conditions are symmetric with respect to the Equator.

The specification of the wind and equilibrium temperature distributions is straightforward; however, specifying the virtual salt flux requires more careful consideration. Neither evaporation nor precipitation is well measured over the ocean, and it is not clear how to specify their distributions for the idealized geometry used here. In order to conserve computational resources, I have used the results from a previous experiment¹¹ with a single-hemisphere version of the model, which was forced with a newtonian-cooling-type condition on salinity as well as on temperature. From the equilibrium solution of this experiment the surface salt flux required to maintain the predicted surface salinity distribution could be computed diagnostically, and is shown in Fig. 1. Note that while the equilibrium salinity profile used in the newtonian-cooling-type condition is zonally symmetric, the implied flux is a function of both latitude and longitude. The magnitude of the flux is largest where transport processes within the ocean (such as advection in the western boundary current) drive the surface salinity furthest from the prescribed values. This salt flux distribution, reflected across the Equator, was then used for all the experiments in the present study.

Initialization

Four experiments are described below, which differ only in their initial conditions. The solution from which the surface salt flux distribution was diagnosed was used as a basic initial condition; however, some problems were experienced in using this initial condition with flux boundary conditions. Had this solution been in exact equilibrium with the surface forcing and perfectly steady, then we would not expect further evolution of the solution after the switch in boundary condition type. For both one- and two-hemisphere versions of the model this was found not to be the case¹¹. When the flux boundary condition was applied to salinity, the pre-existing polar halocline became stronger and spread equatorward. As the halocline spread, the meridional circulation collapsed. This behaviour is not completely under-

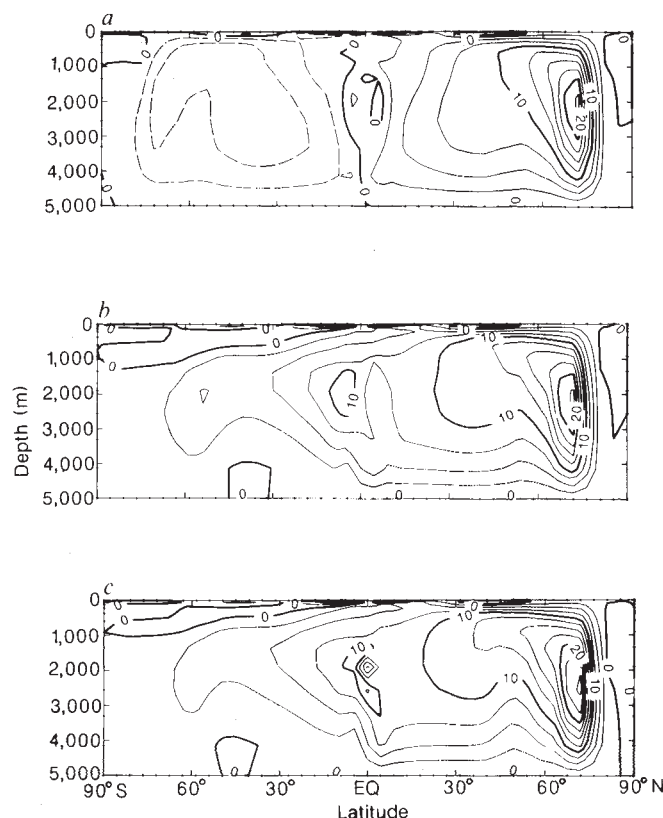


Fig. 3 Stream-function for the zonally integrated meridional overturning circulation for experiment 2 at years 15 (a), 44 (b) and 68 (c). Positive values indicate a clockwise circulation; contour interval, $2.5 \times 10^6 \text{ m}^3 \text{ s}^{-1}$.

stood, but was traced to intermittency in convection near the edge of the halocline. It was found that by adding positive (0.5–2.0‰) salinity perturbations to the high-latitude surface layer (the upper 50 m) of the basic initial condition (effectively moving the halocline away from the main deep water formation region) this behaviour could be avoided. The resulting steady circulation did not differ greatly from that in the unperturbed basic initial condition.

Symmetric circulation

Based on his analysis of a simple hydraulic loop model, Rooth⁸ suggested that an initially equatorially symmetric circulation, forced as described above, would be unstable to infinitesimal asymmetric perturbations and would develop into a pole-to-pole circulation. In order to test this, a control case was initialized with a symmetric state (the basic initial condition was modified by adding positive salinity perturbations of 2‰ to the surface layer poleward of 45° latitude in both hemispheres to prevent the initial halocline instability described above). The model was then integrated forward for >1,300 yr. Although there were some very slight differences in the circulation between the two hemispheres, the solution remained essentially equatorially symmetric for the length of the integration. Figure 2 shows the stream-function for the zonally integrated meridional overturning. The final solution was close to equilibrium, as measured by deep water temperature tendencies and net surface heat fluxes. This implies that the three-dimensional, equatorially symmetric circulation is stable to very small perturbations (arising from numerical round-off error), or that the growth rate of any such instability is extremely small.

Asymmetric circulations

The above results do not rule out the possibility of finite-amplitude perturbations exciting a pole-to-pole mode. The

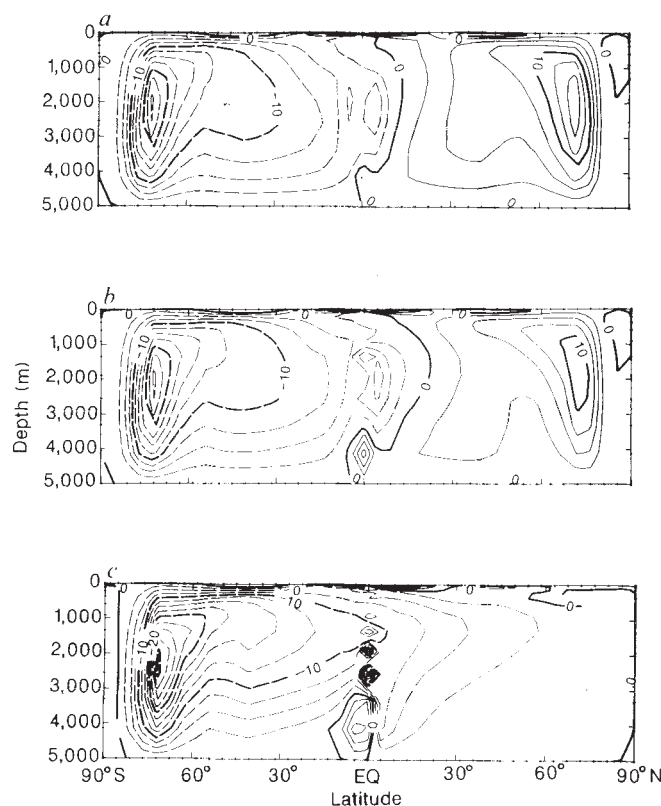


Fig. 4 Stream-function for the zonally integrated meridional overturning circulation for experiment 3 at years 342 (a), 411 (b) and 534 (c). Positive values indicate a clockwise circulation; contour interval, $2.5 \times 10^6 \text{ m}^3 \text{ s}^{-1}$.

second experiment was initialized with an intermediate state of the control case (a nearly converged symmetric circulation), with a negative salinity anomaly of 1‰ added to the surface layer poleward of 45° in the Southern Hemisphere. This experiment was meant to represent the response to a pulse of freshwater input to the surface layer, as might occur during deglaciation. The freshening of the surface layer caused deep convection in the Southern Hemisphere to be interrupted, and hence the residence time of water parcels in the surface layer to increase. With a fixed flux boundary condition, the degree to which the surface salinities are modified depends on the residence time of a parcel at the surface as well as the magnitude of the flux. Thus, cessation of deep water formation (in a region of net precipitation over evaporation) led to further freshening of the surface layers. This is a positive feedback on the initial anomaly, and is part of the mechanism described by Warren⁷ to account for the lack of deep water formation in the North Pacific. In the absence of active deep water formation, the thermohaline circulation in the Southern Hemisphere collapsed. The decreased advection of high-salinity surface water to high southern latitudes by the thermohaline circulation led to a further decrease in the salinity there. The interhemispheric salinity contrast established in this way provided the buoyancy forces needed to drive an interhemispheric circulation, and a pole-to-pole mode was established within ~50 yr (Fig. 3).

The third experiment was initialized with the same solution as the previous case, except that a 2‰ positive salinity anomaly was added to the Southern Hemisphere polar region. The positive anomaly was rapidly mixed vertically by convection, and led to a slight intensification of the meridional circulation in the Southern Hemisphere. As the circulation intensified, it spread slightly across the Equator (Fig. 4a,b). The cross-equatorial circulation caused high-salinity surface water to flow into the Southern Hemisphere at the surface, and low-salinity

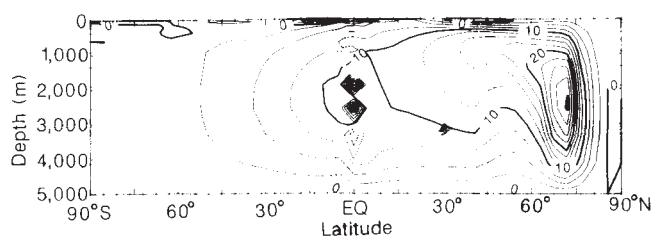


Fig. 5 Stream-function for the zonally integrated meridional overturning circulation for experiment 4 at the end of the integration. Positive values indicate a clockwise circulation; contour interval, $2.5 \times 10^6 \text{ m}^3 \text{ s}^{-1}$.

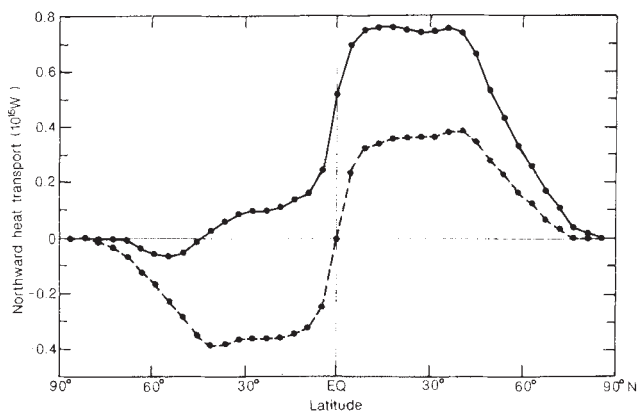


Fig. 6 Northward heat transport for experiment 4 (solid line) at the end of the integration, and for the symmetric solution obtained with newtonian-cooling-type boundary conditions on both temperature and salinity (dashed line).

water to flow out at depth. The net advection of salinity into the Southern Hemisphere is, again, a positive feedback on the initial anomaly. In contrast to the feedback seen in the previous experiment, which was a local interaction between deep convection and vertical salinity differences, the feedback in this case is a global interaction between horizontal salinity differences and the strength of the large-scale circulation. This is the mechanism proposed by Rooth⁸ to provide the buoyancy force to drive the interhemispheric circulation. A pole-to-pole circulation was eventually established (Fig. 4c), but on a much longer timescale than in the case of a negative salinity anomaly.

The final experiment was initialized with the basic initial condition, modified by a positive salinity anomaly added to the Northern Hemisphere polar region. In this case the spread of the Southern Hemisphere polar halocline in the unmodified basic initial condition triggered the pole-to-pole circulation by the same mechanism as in the second experiment. This case was run for >1,200 yrs until a new equilibrium was fully achieved, so that the differences in the climatic properties of the symmetric and asymmetric solutions could be examined. The meridional circulation (Fig. 5) shows that approximately the same amount of deep water forms as in the symmetric case, but the sinking branch is confined to the Northern Hemisphere. The small overturning cells of alternating sign on the Equator (also evident in Fig. 4) are due to inertial instability of the cross-equatorial flow. This type of instability is not scale-selective, and hence appears at the smallest realizable scales in the model (2 grid intervals). Although the instability mechanism is physical, it is not properly resolved by the model and should be parameterized in some way in future studies. The surface salinities in the Southern Hemisphere polar region remain very low, and there is a salinity contrast of ~3‰ between the high latitudes of the two hemispheres. The absence of convection results in very slight surface cooling in the high-latitude Southern Hemisphere.

Thus, the poleward heat transport is dramatically different from that in the symmetric case (Fig. 6). There is equatorward transport over much of the Southern Hemisphere and a cross-equatorial heat flux of the same magnitude as the maximum transport in the symmetric case. Oceanic heat transports of this magnitude could lead to a significant difference between the climates of the two hemispheres.

Discussion

These experiments confirm the hypothesis of Rooth⁸ that inter-hemispheric pole-to-pole circulations can be maintained even under symmetric forcing, but a finite-amplitude salinity anomaly is required to cause an equatorially symmetric circulation to develop into an asymmetric one. At equilibrium, the contrast in salinity between high-latitude regions provides the buoyancy force needed to drive the interhemispheric circulation. The salinity contrast is in turn maintained by the asymmetric thermohaline circulation. The system described here has at least three equilibrium solutions for the same forcing: an equatorially symmetric circulation and two mirror-image pole-to-pole circulations. The response to a negative surface salinity perturbation is rapid and leads to a transition from a symmetric to an asymmetric circulation on a timescale of <100 yr. The response to a positive salinity anomaly, on the other hand, is very slow, leading to a transition from a symmetric circulation to an asymmetric circulation over many hundreds of years. The pulse-like nature of the perturbations used in this study prevent these results from being directly compared to the oceanic response to salinity changes caused by glacial buildup or collapse, which occur over relatively long timescales. Nevertheless, an under-

standing of the mechanisms which were identified in these experiments will be useful in interpreting the changes in ocean circulation that occur during these climatic transitions.

These experiments provide a first look at the complicated behaviour which can result from the interaction of the salinity distribution and the thermohaline circulation. They were designed to illustrate only processes operating within the ocean, and many other processes which are important in the climate system have been neglected. In particular, one would like to know whether multiple equilibria may still exist for asymmetric surface forcing or basin geometry (as occur in the real world), and to determine the processes involved in the transition from one asymmetric state to another (rather than from the symmetric state to an asymmetric state, as modelled here). Also, the large changes in sea surface temperature between the different solutions would be expected to cause changes in the atmospheric forcing, especially in the evaporative fluxes, which were held constant in these experiments. Future studies should investigate the robustness of these results with respect to changes in basin geometry and surface forcing, and the inclusion of neglected features, such as sea ice and interactive atmospheric circulations. The results of such a study should help to answer the question posed by Broecker *et al.*⁴: "Does the ocean-atmosphere system have more than one stable mode of operation?"

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Novel subunit-subunit interactions in the structure of glutamine synthetase

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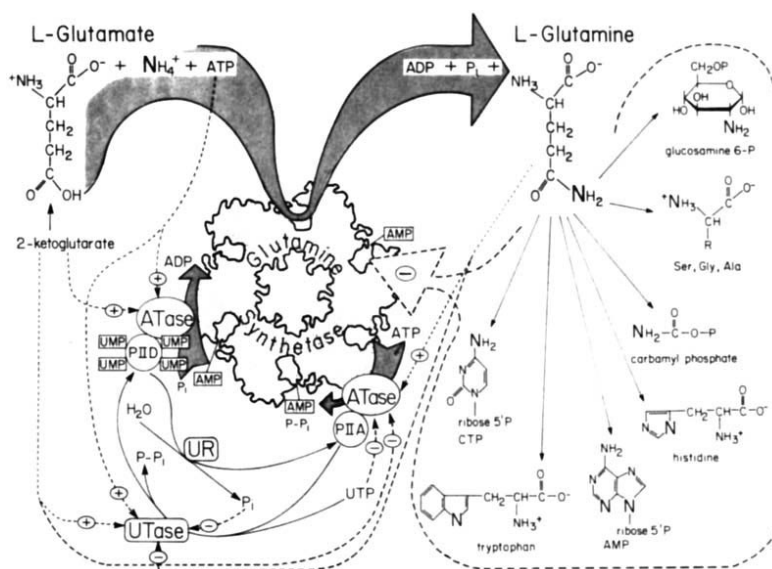
We present an atomic model for glutamine synthetase, an enzyme of central importance in bacterial nitrogen metabolism, from X-ray crystallography. The 12 identical subunits are arranged as the carbon atoms in two face-to-face benzene rings, with unusual subunit contacts. Our model, which places the active sites at the subunit interfaces, suggests a mechanism for the main functional role of glutamine synthetase: how the enzyme regulates the rate of synthesis of glutamine in response to covalent modification and feedback inhibition.

THE large size (relative molecular mass, $M_r = 12 \times 51,628$) and complex pattern of regulation of bacterial glutamine synthetase (GS) stem from its central role in cellular nitrogen metabolism¹⁻³. It catalyses the condensation of ammonia with glutamate to yield glutamine, which in turn is a source of nitrogen in the biosynthesis of many metabolites, eight of which act as feedback inhibitors on GS (Fig. 1). GS is also regulated by covalent modification⁴; when glutamine is abundant, tyrosyl residue 397

is adenylylated, which increases the sensitivity of GS to most feedback inhibitors. During the course of its normal function at least 15 known substrates, inhibitors, metals and proteins bind to GS. We seek to understand how these 15 ligands are linked in the control of synthesis of glutamine.

Eighteen years ago, structural studies by electron microscopy revealed that the 12 polypeptide chains of bacterial GS are arranged in two rings of six⁵, with 622 point-group symmetry.

Fig. 1. Interactions of glutamine synthetase¹⁻³. The ATP-dependent condensation of ammonia with glutamate is shown catalysed at one active site of the GS molecule. The amide nitrogen of glutamine (large N) is a source of nitrogen for the metabolites shown to the right in which large Ns label nitrogens derived from the amide group of glutamine⁴⁶. Each of these end-products is a feedback inhibitor of GS⁴⁷ acting at separate sites. Feedback inhibition is represented at the right by the heavy dashed arrow with a negative sign, acting on GS. The active site which these inhibitors affect is shown with a nearby AMP (adenylyl) moiety covalently bound to the enzyme. GS subunits are covalently modified by adenylation through a phosphodiester linkage to a specific tyrosyl hydroxyl⁴, Tyr 397 in the sequence of GS from *S. typhimurium*²². Adenylation is catalysed by the enzyme adenylyltransferase (ATase) aided by a tetrameric regulatory protein PII (refs 48, 49). This reaction is shown at the lower right of the GS molecule, where the ATase-PII complex converts ATP and an unadenylylated subunit site into an adenylylated subunit and pyrophosphate. Deadenylation is shown at the left side of the GS molecule. Removal of the adenylyl groups is catalysed by the same two proteins, but for removal PII is modified by uridylation. Uridylation of PII is catalysed by transesterase (UTase), shown at the bottom left of the figure. Hydrolytic removal of the UMP groups from PII is catalysed by the uridylyl removing enzyme (UR). These three levels of enzymatic catalysis in the formation of glutamine respond sensitively to concentrations of metabolites². For example, high concentrations of glutamine and inorganic phosphate (P_i) inhibit UTase, with the effect of increased adenylation of GS, leading to more effective inhibition of GS by the multiple end-products of glutamine shown to the right, and decreased formation of glutamine. Inhibition by effectors is represented by heavy dashed lines with negative signs; activation by light dashed lines with positive signs.



Later a low-resolution, three-dimensional reconstruction of GS was produced by image processing of electron micrographs of helical cables of GS⁶. The variability of the cables suggested that the GS subunit is composed of folding regions connected by a flexible portion.⁷ Also the general region of the adenylyl moiety was mapped by immunoelectron microscopy at the outer enzyme surface⁸. Here we report the determination of an atomic model for the 5,616 amino-acid residues of the GS molecule and suggest a function for the subunit interface in catalysis and regulation.

Structure determination

Crystals and data collection. GS is one of the largest and most complex enzyme structures yet determined. Unusual aspects of the structure determination include: (1) collection of all data by an area detector⁹; (2) initiation of phase determination with a metal complex containing 450 electrons¹⁰; and (3) phase refinement by 12-fold averaging of electron density.

We used crystal form XIV of completely unadenylylated GS from *Salmonella typhimurium*¹¹, of space group C2 and unit-cell

dimensions $a = 235.5 \text{ \AA}$, $b = 134.5 \text{ \AA}$, $c = 200.1 \text{ \AA}$; and $\beta = 102.8^\circ$. In the asymmetrical unit of the crystal there is one full GS dodecamer of $M_r = 620,000$. Determination of the molecular orientation and position in the unit cell was essentially as described earlier for *E. coli* GS form III^{12,13}, which is inferior but effectively isomorphous with this form. A minor difference here is that we added 1 mM MnCl_2 to the preparation buffer and omitted dithiothreitol throughout.

We collected data for the native enzyme and K_2PtCl_4 (Pt) derivative to 3.5 $\text{\AA}$ resolution and for a $\text{Nb}_6\text{Cl}_{14}$ (Nb) derivative¹⁰ to 6 $\text{\AA}$ resolution (see Table 1). There are 64,005 unique reflections in the final 3.5- $\text{\AA}$ resolution electron-density map.

Location and refinement of heavy atoms. We located Nb and Pt complexes at 10 $\text{\AA}$ resolution by the difference Patterson search procedure of Argos and Rossmann^{14,15} and through 622 symmetry-averaged cross-difference Fourier maps¹⁶. The best solution for the Nb search (using intra- and intermolecular vectors and multiplicity corrections) was refined using the origin-removed difference Patterson method¹⁷. From the refined parameters, we calculated single isomorphous replacement phases

Table 1 Data collection

Crystal†	Compound	Resolution		Reflections	Unique reflections	Rejects	R_{sym}^* (%)	$\langle \Delta F \rangle / \langle F \rangle$
		min	max					
GSS010	Native	7	3.5	112,592	65,233	393	5.5	—
GSS011	Native	54	5.8	63,441	—	—	—	—
GSNb02	$\text{Nb}_6\text{Cl}_{14}$	38	4.8	35,355	13,696	117	10.2	0.33
GSPT02	K_2PtCl_4	67	5.3	17,992	—	—	—	—
GSPT03	K_2PtCl_4	60	5.3	16,224	10,125	114	10.7	0.21
GSPT04	K_2PtCl_4	7	3.5	140,165	48,905	7	10.1	0.14
GSMS01	MetSox‡	54	3.5	237,488	69,569	12,229	10.2	0.51

For data collection, the Elliott GX-6 X-ray generator was generally run at 40 kV and 50 mA. A data-collection 'frame' was usually 0.1° with the time per frame varying between 20 and 75 s. The total rotation angle was as small as 152° (for crystal GSPT02) and as large as 988° (in 13 orientations for GSMS01, an extremely radiation resistant crystal). The crystal-to-detector distance was 1,046–1,421 mm.

* R_{sym} = the conventional discrepancy R -factor for scaling of symmetry-related intensities after rejecting the number of 'outlying' reflections given in the rejects column.

† Crystals varied in size from 0.1 mm on an edge for GSPT03 to $0.67 \times 0.42 \times 0.25 \text{ mm}^3$ for GSS010.

‡ GS reacted with MetSox, Mn^{2+} and ATP before crystallization⁴⁵.

§ There are 76,950 unique reflections to 3.5- $\text{\AA}$ resolution. Of the 64,005 in the final map, 93% have $F/\sigma > 2$.

|| Mean fractional change in structure-factor magnitude produced by the ligand.

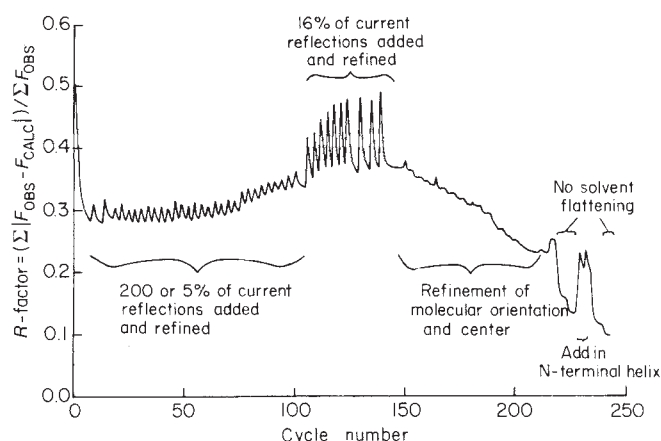


Fig. 2. Phase refinement. The residual R -factor, a measure of the consistency of the electron density with experimental structure factors, shown as a function of the refinement cycle. Each cycle consists of calculation of the electron density from observed structure-factor magnitudes and current phases; 12-fold averaging of the GS molecule within a defined molecular envelope according to the current orientation and position; replacement of four averaged molecules within the C2 unit cell; (for some cycles) replacing each density element outside the molecular envelope by the average of all (solvent flattening); and Fourier inverting to obtain calculated structure factors for computing the R -factor and phases for computing the electron density for the next cycle. At most stages, calculated structure factors were included in electron-density maps for reflections which were not measured⁵⁰, but were not included in R -factors. The R -factor for the initial multiple isomorphous replacement phases at 10 Å is 0.51. Eight cycles of refinement lowers R to 0.28, similar to the value expected for random phases⁵¹. Addition of 200 reflections at resolution just finer than 10 Å increases R to 0.31, and in three further cycles reduces R again to 0.28. The pattern to cycle 100 was to add 200 reflections (later 5% of the current number of reflections), followed with three cycles of refinement. After cycle 105, we added 16% of current reflections, followed by refinement. After cycle 145 each few cycles of phase refinement were interspersed by Fourier refinement of the molecular orientation. At cycle 200, we computed an electron-density map; we traced much of the polypeptide backbone but there are at least three 'ends' and the chain density is weak at the molecular boundaries. At cycle 216 the procedure of substituting calculated structure factors for unobserved reflections was temporarily abandoned, leading to an increase in R . Then at cycle 219 solvent flattening was halted, producing a marked drop in R . At cycle 226 the protein map was again inspected. The continuity and clarity were improved, and a helix of 12 residues, previously outside the envelope, was discovered at the N-terminus and added to the molecular envelope at cycle 228. Refinement continued to cycle 234 ($R = 0.200$, with solvent flattening) and to the final electron-density map at cycle 241 ($R = 0.097$, no solvent flattening).

and a 622 symmetry-averaged Pt cross-difference Fourier. We found 2 sets of 12 sites significantly above the noise level, with the major set corresponding to the second-best solution from the Pt difference Patterson vector search. In confirmation, phases calculated from this 2 site per subunit Pt model reproduce the single site per subunit Nb model in a 622 symmetry-averaged Nb cross-difference Fourier. Using 6-Å multiple isomorphous replacement phases, the averaged difference Fourier map for Nb is free of additional sites, but the Pt difference map reveals one additional set of sites (Pt-3 in Table 2). A final cycle of heavy-atom refinement gives the results in Table 2 and the starting phases for symmetry-averaged phase refinement. The molecular orientation and position were determined from a least-squares fit of all 48 heavy atoms to 622 symmetry, weighted by the relative group occupancy.

Phases were refined with a local implementation of the Bricogne¹⁸ algorithm of molecular replacement¹⁹ for real-space averaging of the 12 subunits (Fig. 2). Real-space averaging was

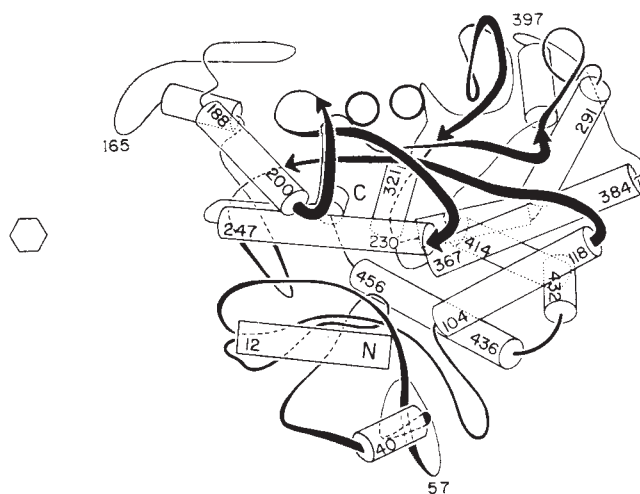


Fig. 3 A schematic drawing of the GS polypeptide chain. The 6-fold axis is indicated by a hexagon at the left, and the metal ions by two circles in the upper centre. Cylinders, α -helices; heavy arrows, six prominent β -strands surrounding the metals. Bottom left, the N-terminal domain (residues 1–103). The junction between the N- and C-terminal domains is the start of the third helix (residue 104). The central loop is at the upper left. Diagram based on a program written by A. M. Lesk and K. D. Hardman⁵².

Table 2 Heavy-atom refinement

Heavy atom	Resolution	Site	Occupancy*	B†	Position‡	$f_H/E\%$
Nb	10	Nb-1	78.9 ± 31.1	150	2.1	1.57
Pt	10	Pt-1	31.3 ± 23.0	50	4.1	1.57
		Pt-2	13.6 ± 5.2	50		
Nb/Pt	6	Nb-1	94.3 ± 20.7	150		1.70
		Pt-1	39.3 ± 7.7	50	1.5	
		Pt-2	18.8 ± 8.3	50		1.88
		Pt-3	25.2 ± 7.8	50		

* Occupancy on an arbitrary scale $\pm$ the r.m.s. deviation of occupancy at the 12 equivalent sites of one GS molecule.

† Temperature factor in Å², determined from a Wilson plot.

‡ R.m.s. deviations (Å) of the 12 sites from equivalent sites obeying 622 symmetry.

§ Ratio of calculated heavy-atom structure factor to r.m.s. lack-of-closure error, a measure of signal-to-error for the heavy-atom derivative.

supplemented at most stages by solvent flattening^{20,21} and occasionally by reciprocal space refinement of the molecular orientation and position (R.J.A., manuscript in preparation).

Atomic model. An atomic model was constructed by fitting the amino-acid sequence²² to the averaged electron density with the computer program BILDER²³, modified by R. C. Ladner. Although the electron density is at a nominal resolution of only 3.5 Å, the path of the polypeptide backbone is clear (with conceivable bifurcations at only two points). There are only two termini, and the direction of the chain is evident at the eight long α -helices. The fit of side chains to the electron density is generally good, but there are three exceptions: Pro 447 appears much more like Arg; the sequence Met 65–Val 66–Leu 67 appears more like Leu–Met–Val; and the region from Gly 267 to Cys 270 is difficult to fit. Small side chains are generally indistinguishable from each other. Also suggesting a correct placement of the amino-acid sequence within the electron density are: (1) reasonable metal ligands His, Glu and Ser; (2) hydrophilic residues in regions exposed to solvent, and hydrophobic residues in buried regions; (3) all but one turn or loop contain Pro and/or Gly; (4) most α -helices predicted by the Lim²⁴ algorithm overlap observed α -helices; (5) buried carboxy-termini, consistent with the observation¹ that GS is resistant to carboxypeptidase attack;

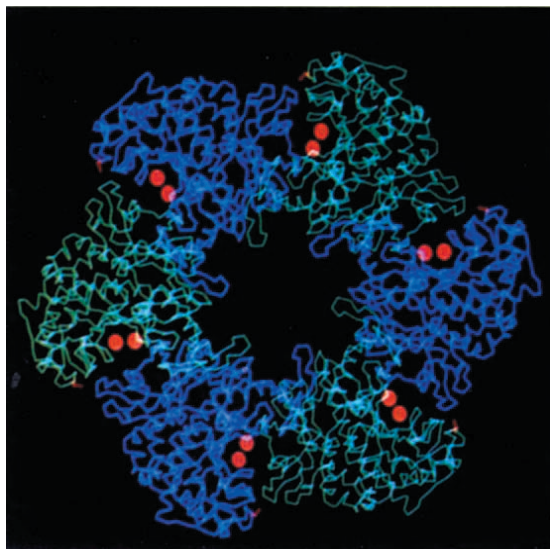


Fig. 4 The GS molecule (upper half) projected down the 6-fold axis of symmetry. The six subunits of the upper ring of the molecule are shown as line segments connecting sequential α -carbon atoms. The outer diameter of the molecule, including side chains, is 143 Å. Six active sites are marked by the pairs of Mn^{2+} ions (red). Six 2-fold axes of symmetry lie in a plane parallel to the figure below these subunits, and relate these six to six subunits below. The six subunits of the lower ring (not shown) are roughly eclipsed with the subunits above, whereas the active sites are staggered. The side-chain of Tyr 397 (red) is between subunits at larger radius than the active site. This is the adenylation site.

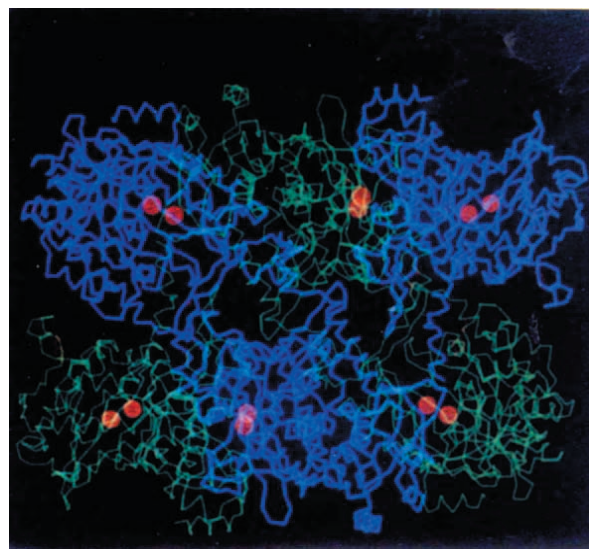
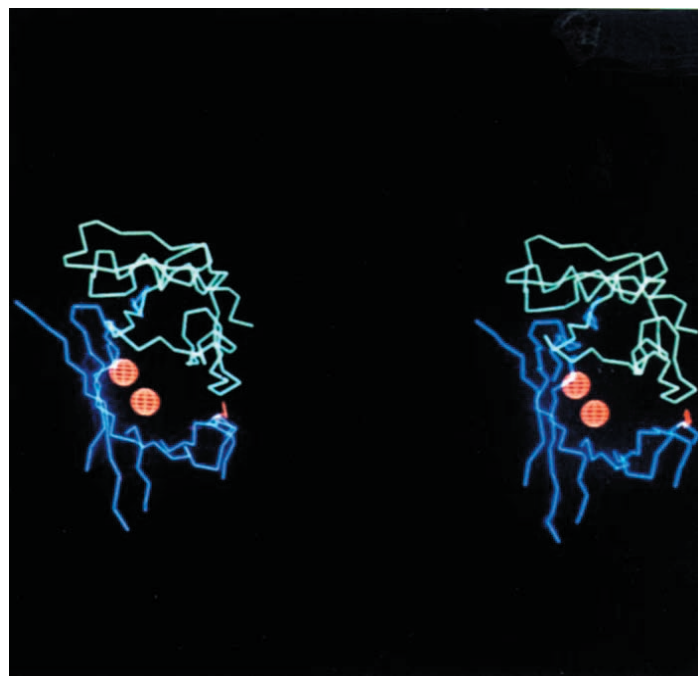


Fig. 5 The GS molecule (front half) viewed from the side, down one of the 2-fold axes. Only the six nearest subunits are shown. The 6-fold axis is vertical. The molecule extends 103 Å (including side-chains) along the 6-fold axis. Note that each plane of Mn^{2+} ions is about midway between the top (or bottom) of the GS molecule and the central plane of the 2-fold axes. At the centre there is a four-strand β -sheet formed by the β -loops of the two central subunits, along with two C-terminal helical thongs of these two subunits.

Fig. 6 Stereo view of the active site. Orientation is that of the lower right subunit of Fig. 4, with the outside of the GS molecule to the right and the central channel to the left. The view is down the 6-fold axis, in the same direction as Figs 3 and 4. The Mn^{2+} sites are shown in the active-site channel formed by strands of β -structure from the C-terminal domain (blue) of one subunit and from the N-terminal domain (green) of a second subunit. The side-chain of Tyr 397 is shown at the lower right, extending from the C-domain towards the neighbouring N-domain. The two Mn^{2+} ions each accept four ligands from the C-domain. In addition, there is at least one side chain in the N-domain (Asp 50) that is directed towards a Mn^{2+} ion, but is beyond the distance of ligation. Segments of polypeptide chain are as follows: the N-domain segment shows residues 1-91; the five segments shown from the C-domain are residues 122-136, 207-227, 262-275, 342-360 and 391-399. There is also a short segment under the N-domain consisting of residues 176-180. Residues 31-34 of the N-domain seem to form an antiparallel β interaction with residues 207-210 of the C-domain. The diameter of the β -barrel is ~ 10 Å, compared with 10-20 Å for the axes of hyperboloids fit to β -barrels of other proteins⁵³.



(6) all PtCl_4^- ions are bound to Met residues²⁵; and (7) the atomic *R*-factor computed for the model for all 43,656 non-hydrogen atoms and all 64,005 reflections is 0.511, acceptable for so large a structure before atomic refinement.

We now go on to describe the atomic model, with emphasis on the subunit interfaces and their possible functions. We use 'up' to mean higher values of the coordinate *z* along the 6-fold axis of symmetry; 'down' for smaller absolute values of *z*, towards the origin at the intersection of the 6-fold axis with the

plane of the six 2-fold axes; 'in' to mean towards the 6-fold axis; and 'out' to greater radial distances from the 6-fold axis. For clarity, most figures show only segments connecting the α -carbon atoms plus metals.

The structure

Subunit fold. The folding of the polypeptide chain in a single subunit is shown schematically in Fig. 3. The small N-terminal domain (residues 1-103, the N-domain), formed from six strands

of antiparallel β -sheet and one large and one small α -helix, is at the bottom of Fig. 3 and is connected to the large C-terminal domain (C-domain) just before the long helix starting at residue 104, but by no other covalent link. At the top of Fig. 3, six prominent strands of β -structure from the C-domain form a partial barrel around the two metal ions. These strands tend to run parallel to the 6-fold axis (normal to the plane of Fig. 3). Separating these strands from the N-domain of the same subunit is the central region of the subunit, which is mainly α -helical. The α -helices tend to run perpendicular to the direction of the β -strands (that is, they are roughly in the plane of Fig. 3). Thus the C-domain in GS contains many α -helices (7 long and 6 short) and an extended β -sheet (6 long and 3 short strands) but is unlike many other enzyme domains with mixed α - and β -segments²⁶, including nucleotide-binding domains²⁷; in GS the β -strands are antiparallel and the helices run perpendicular to the β -strands.

Special functions seem to be fulfilled by some of the 25 turns and loops. One of these is the Trp 57 loop (Fig. 3, bottom; Fig. 6, right) in the lowest part of the N-domain, donating two β -strands (residues 44–52 and 59–70) that form the far side of the active site β -barrel (see below). That is, this loop is next to the active site β -strands of the neighbouring subunit in the same ring of six. At the bottom of this loop is Trp 57, 6 Å from Tyr 397 in the C-domain of the neighbouring subunit. A second 'central loop' is of possible functional importance (Fig. 3, upper left), and is discussed below. The ' β -loop' lies back along the chain from the central loop, extending downwards parallel to the 6-fold axis and making contact with the β -loop of the subunit below. The two β -loops form a four-stranded sheet. Finally, there is an extended segment of polypeptide chain at the molecular surface, residues 384–399 containing Tyr 397 (Fig. 3 upper right), the residue that can be adenylylated^{22,28}.

Molecular shape. The view of GS down the 6-fold axis (Fig. 4) reveals a petal-shaped molecule with outer diameter of 143 Å. As anticipated from electron micrographs^{5,6,29} there is a central channel ~40 Å in diameter, but it is not completely open. The central loop protrudes into the channel, so that the open inner diameter is only 24 Å. This loop, formed by the hydrophilic segment of residues 156–173, and the following segment are sensitive to various proteases^{30–32}. Four different proteases cleave the GS chain into two major segments. To emphasize that such 'domains' detected by proteolysis are not necessarily the contiguously folded regions of polypeptide termed 'domains' by crystallographers, note that the central loop is not at the boundary of the two folding domains (residue 103).

Six active sites are visible in Fig. 4, marked by the pairs of Mn^{2+} ions. These active-site channels are roughly midway between the outer periphery of the GS molecule and the central channel. Each active site is at the interface between two subunits related to each other by the 6-fold axis of symmetry (see below).

Figure 5 shows the view from the side of the molecule down one of the 2-fold axes. Three N termini are visible at the top of the molecule and three at the bottom: these are the nearly horizontal α -helices. The C terminus is at the end of a helix that crosses the central plane containing the six 2-fold axes and penetrates into the subunit in the opposite ring. Other than these 12 'helical thongs', the only significant contact between subunits across the plane of six 2-fold axes is at the β -loop, which with an identical β -loop from the subunit below, forms a small, four-stranded β -sheet.

Discussion

The active site. The GS active site is marked in the electron-density map by two unmistakable features. First, the two metal ions required for catalytic activity are the strongest features. They are spherical and each is connected to the protein backbone by four ligands. The Mn–Mn separation is 5.8 Å, exactly the distance determined from spectroscopic studies³³. Second, the transition-state analogue L-methionine-S-sulphoximine (Met-

Sox)^{34,35} difference map marks the active site. Also tending to confirm that this region is the active site is the observation that the Met-His-Cys-Met sequence (subject to oxidation with loss of catalytic activity³⁶) is one of the metal–ligand sequences.

The region marked by the two metal ions and MetSox is a cylindrical channel, roughly parallel to the 6-fold axis and open at the top of the molecule. Six such channels are evident in Fig. 4, and one is shown in greater detail in Fig. 6. The metal ions are on one side of the channel, nearer to the C-domain to which they are bound. They lie 28 Å under the top of the molecule, and 14–22 Å below the irregular mouth of the channel. The major MetSox difference density is above the metals and the most direct access from solution seems to be down the channel from the top. The channel also opens at the bottom of the subunit near the plane of 2-fold symmetries.

A striking aspect of the active site is that it is formed by two polypeptide chains. In this sense, and also in that both of its surfaces are formed by antiparallel β -structure, it is similar to the antigen-binding sites of antibodies. One surface of the GS active site is formed mainly by 6 β -strands of the C-domain of one subunit, and the other surface by two β -strands of the N-domain of the adjacent subunit. This proximity of the N-terminal region to the active site is consistent with the chemical labelling study of Pinkofsky *et al.*³⁷, who found that binding the ATP analogue 5'-*p*-fluorosulphonylbenzoyladenine at the active site modifies Lys 47. This residue is in the Trp 57 loop that lies across the active-site channel from the metal ions, presumably next to the ATP site. (However, the side chain of Lys 47 is ~13 Å above the centre of the MetSox site. This suggests that either: (1) the ATP analogue is displaced from the MetSox site when it reacts with Lys 47; (2) through a conformational change Lys 47 approaches 13 Å closer to the metals; or (3) our model is incorrect by several residues in the segment of polypeptide containing Lys 47.) Although the formation of an active site from the intimate association of two subunits is unusual, it has already been found in studies of aspartate amino transferase³⁸, glutathione reductase³⁹, aspartate transcarbamylase⁴⁰ and catalase⁴¹.

The distance between active sites in GS is of interest for interpretation of cooperative effects^{1,42}. Neighbouring active sites within the same ring of six subunits are separated by about 45 Å (measured from the point midway between two metals). The smallest separation of active sites in opposite layers of six subunits is 52 Å. These separations are smaller than those suggested by fluorescence-energy transfer⁴², which are 56–60 Å between nearest nucleotide probes in the same ring of subunits and 60–78 Å between nearest probes in opposite rings.

The adenylylation site. Adenylylation of GS alters many of its properties^{1–3}. Although the enzyme in our crystals is completely unadenylylated, it is known that Tyr 397 is adenylylated on its phenolic hydroxyl^{22,28,43} (see Figs 4, 6). It is at the end of an extended, exposed segment of polypeptide at the outer surface of the molecule, a location that is compatible with the one indicated by immunoelectron microscopy⁸ and with a solvent-exposed position suggested by spectroscopic studies⁴⁴. The phenolic hydroxyl in our model is ~22 Å from the point between the two metals. This separation is considerably greater than that suggested by nuclear magnetic resonance and fluorescence measurements for the distance of the metals from the adenylyl moiety⁴⁴. Conceivably the difference in X-ray and spectroscopic results can be accounted for by a protein conformational change on adenylylation, which brings the adenylyl group closer to the metals. Also the phenolic hydroxyl projects upwards and inwards, so that the adenylyl group is probably closer to the metals than is the tyrosyl hydroxyl.

Subunit contacts. The unfolding and refolding of *E. coli* GS has been studied extensively^{1,45}. Extraction of the metal ions produces a relaxed enzyme that is susceptible to subunit separation by mild alkali or low concentrations of urea¹. It has also been found⁴⁵ that side-to-side subunit contacts are more labile than

up-to-down subunit contacts. These observations can be explained by the structure in Figs 3–5. Some of the side-to-side contacts of subunits occur between the N- and C-domains of the neighbouring subunits in the region of the active-site channel. Removal of the metal ions might be expected to be a disorganizing influence on the eight β -strands around the channel.

Our finding that the active site is at the interface of subunits related by the 6-fold axis is consistent with studies of the stabilization of GS oligomers by MetSox. Maurizi and Ginsburg⁴⁵ found that GS that had been complexed with MetSox resists cleavage of intra-ring subunit contacts in 6 M guanidine hydrochloride. This observation can be explained in terms of the structure of Fig. 4 if MetSox strengthens the interaction of the N-domain of one subunit with the C-domain of the neighbouring subunit at the active site. Maurizi and Ginsburg could isolate stable oligomeric intermediates containing 4, 6, 8 and 10 subunits, but not 2 subunits, in samples that had been treated with substoichiometric amounts of MetSox. This is to be expected if MetSox stabilizes the side-to-side association of two pairs of subunits, each member of the pair linked top-to-bottom.

Top-to-bottom subunit contacts involve two striking features of the GS chain, both visible in Fig. 5: first, the helical thong at the C-terminus, which crosses the plane of the 2-fold axis and is inserted into the subunit on the other side; and second, the small four-stranded β -sheet formed from the two strands of the β -loop projecting downwards and binding at a 2-fold axis of symmetry to the β -loop projected upwards from the subunit below.

Long-range communication. The central loop (residues 156–173) and the segment following it are sensitive to several secreted proteases with various residue specificities^{30–32}. Proteolytic cleavage of the GS chain in this region produces a nicked GS that no longer binds MetSox. Conversely, binding of the substrate glutamate tends to protect this loop from proteolysis, although one proteolysis site (Glu 165) is 35 Å from the active site. Also, some of the feedback inhibitors offer limited protection from proteolysis: apparently the central loop can communicate with the active site and with the inhibitor binding sites. This pathway of communication could involve Tyr 179, whose side-chain extends towards the inner metal ion.

Catalysis and regulation. The model presented here for GS in its unadenylylated state, free of substrates and inhibitors but

bound to Mn^{2+} ions, does not answer many questions about the structural basis of catalysis and regulation. But it does lead to several speculations. The catalytic cycle must involve cooperation of the C-domain of one subunit with part of the N-domain of its neighbour around the 6-fold axis. Movement of the Trp 57 loop of the N-domain relative to the neighbouring C-domain, thereby enclosing the active site more closely, seems probable. There is indirect structural evidence for some domain movement from analysis of the variation of the structures of cables of GS molecules⁷. From the observed variation in twist of the cables, it was postulated that GS contains two structural segments that can move relative to each other by a distance of the order of 4–10 Å. In our present model, it seems likely that contacts between GS molecules in the cables involve the N-domains on neighbouring molecules, suggesting that part of the N-domain can move relative to the C-domain, either opening or restricting the region of the metal ions. If the Trp 57 loop moves closer to the C-domain than it is in our model, Asp 50 will approach the outer metal more closely, and perhaps participate in the catalytic process. Fluorescence energy transfer provides additional evidence⁴² of conformational change near the active site during formation of cables.

How does adenylylation increase the affinity of feedback inhibitors for GS and modulate other aspects of catalytic activity¹? Although there is no direct evidence on this question, note that Tyr 397 on the C-domain is near Trp 57 on the N-domain: the side chains are separated by about 6 Å in our model. It is possible that the adenylyl group interacts with Trp 57 or other N-domain residues, restricting the structure or motion of part of the N-domain with respect to the C-domain. Because the active-site channel is formed at the junction of the N- and C-domains, such a restriction could affect catalysis or binding. This restriction could be the closing of the active site (producing a taut GS), or alternatively, adenylylation of Tyr 397 could prevent the N-domain from making a full excursion to the optimal transition-state structure. Further study of the MetSox complex and other GS complexes should elucidate these questions.

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Determining the Hubble constant from gravitational wave observations

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I report here how gravitational wave observations can be used to determine the Hubble constant, H_0 . The nearly monochromatic gravitational waves emitted by the decaying orbit of an ultra-compact, two-neutron-star binary system just before the stars coalesce are very likely to be detected by the kilometre-sized interferometric gravitational wave antennas now being designed¹⁻⁴. The signal is easily identified and contains enough information to determine the absolute distance to the binary, independently of any assumptions about the masses of the stars. Ten events out to 100 Mpc may suffice to measure the Hubble constant to 3% accuracy.

The signal from a system of two $1M_\odot$ stars (where $M_\odot$ is the mass of the Sun) will sweep from 100 Hz to 1 kHz in ~ 3 s. There might be three events per year out to 100 Mpc, and if the detectors achieve their current design sensitivity, such events will be detectable with a signal-to-noise ratio of 30. To determine the distance, the signal has to be observed by a worldwide network of three, and preferably four, detectors. By measuring both the response of the detectors and the delays between the arrival times of the signal at different detectors, the network should be able to locate the source in an error box of ~ 36 square degrees. There is some chance that the coalescence event will be optically identifiable (I.D. Novikov, personal communication); otherwise, clustering of galaxies provides a statistical method that will still yield H_0 after remarkably few events. Here I give only a brief discussion; full details will be published elsewhere.

Several detectors being developed in the United States and Europe²⁻⁴ will take the form of interferometers with arm lengths 1–4 km, observing bandwidths 10^2 – 10^4 Hz and r.m.s. noise levels at 100 Hz of $<10^{-24}$ strain $\text{Hz}^{-1/2}$. Within 10 years we may expect that there will be four or five such detectors in operation in America and Europe, with typical separations of 6,500 km. [It is possible that bar detectors could contribute to these observations. However, because of their narrow bandwidth, their detection of coalescing binaries requires quite different methods, which have not been studied. I shall therefore concentrate on interferometric detectors.]

Although there are many possible sources of gravitational waves, the most promising for detection by these instruments seems to be the coalescence of binary neutron stars, as will happen to the binary pulsar PSR1913+16 in $\sim 10^8$ yr. The gravitational waves from these sources before coalescence can be predicted very reliably (K. S. Thorne, personal communication). As an orbit decays through the emission of gravitational radiation, its eccentricity is reduced, so we need only consider systems with circular orbits⁵. Consider a binary at a distance $100r_{100}$ Mpc, with total mass $m_T M_\odot$ and reduced mass $\mu M_\odot$, emitting waves at frequency $100f_{100}$ Hz (twice its orbital frequency). The standard quadrupole formula^{6,7} of general relativity shows that the waves will have amplitude (r.m.s.-averaged over detector and source orientations)

$$\langle h \rangle = 1 \times 10^{-23} m_T^{2/3} \mu^{2/3} f_{100}^{-1/3} \quad (1)$$

and that their frequency will change on a timescale

$$\tau = f/\dot{f} = 7.8 m_T^{-2/3} \mu^{-1} f_{100}^{-8/3} \text{ s} \quad (2)$$

Two $1.4M_\odot$ neutron stars will coalesce¹ when $f = 10^3$ Hz. By using matched filters to analyse the data⁵, the noise can effectively be limited to a bandwidth of $\sim \tau^{-1}$. This will enable

the detectors to see binary neutron star sources at 100 Hz at a distance of 100 Mpc, with a mean signal-to-noise ratio (SNR) of >30 . An observation will therefore determine τ and h to perhaps 3%. The key to our method is that the stars' masses enter equations (1) and (2) in exactly the same way, so that

$$r_{100} = 7.8 f_{100}^{-2} (\langle h_{23} \rangle \tau)^{-1} \quad (3)$$

where $\langle h_{23} \rangle = \langle h \rangle \times 10^{23}$, independently of the masses of the stars.

This result is not quite so strong as it seems, as equation (1) gives the r.m.s. value of h averaged over orientations, whereas the value of h inferred from the network's observations will depend on the binary system's orientation and position relative to the detectors as well as its distance. However, these can be determined from the observations: as I show below, provided that three or more detectors register the same event, they can determine the location on the sky and the degree of elliptical polarization of the wave. (In general relativity, gravitational waves are transverse and have only two independent polarizations⁶⁻⁷.) Now, the radiation emitted by the binary along its angular momentum axis is circularly polarized, whereas that in the equatorial plane is linearly polarized. The degree of elliptical polarization therefore determines the inclination of the orbit to the line of sight, which enables us to solve for r_{100} in terms of the observed h . Equation (3) also depends on being able to model the system as two newtonian point masses. As we shall see below, tidal and relativistic corrections are negligible in the range of orbital parameters we require.

Being able to determine r directly from the observations is remarkable in itself, but it is only really useful if the source of the event can be identified. For this an accurate position is required. Because this accuracy is crucial for the determination of the Hubble constant, I will discuss it in some detail.

Each detector has quadrupolar linear polarization, so it is not highly directional; however, the differences in arrival time of a wave at different detectors can be used to triangulate the position. Between any two detectors with separation d , a wave travelling at an angle θ to the line joining the detectors will arrive at the second detector with a delay $\Delta t = d \cos \theta / c$ relative to the first, where c is the speed of light. For $d \approx 6.5 \times 10^3$ km, we have $|\Delta t| \leq 22$ ms. As the two detectors will generally not have the same polarization, there will be a further effective time delay due to the wave's elliptical polarization. Such a polarization can be regarded as a superposition of the two independent linear polarizations defined by the detectors, with a phase shift between them. This phase shift means that differently polarized detectors record the wave train with extra time delays of up to one period ($+10$ ms for a 100-Hz signal). The two independent time delays measured among three detectors and the three measured amplitudes are sufficient to determine the waves' five unknowns: arrival directions (two), amplitudes of the different polarizations (two), and phase lag of the polarizations (one).

The precision with which the source's position and polarization can be measured depends on the two sorts of errors: the accuracy with which the arrival time of the wave at a detector (and hence the time delays) can be determined, and the accuracy with which the amplitude of the detector's response can be measured. In what follows, I will assume that $m_T^{2/3} \mu = 1$ (for example, two stars of $\sim 1.1M_\odot$) to illustrate the situation. We shall see that the timing accuracy is typically 1% of the maximum timing range (from -22 to $+22$ ms), and the amplitude error is $\sim 3\%$. When only three detectors see an event, there are actually two error boxes of size $10^\circ \times 10^\circ$, which may be too large for our purposes. I will therefore consider events detected in four instruments. The seven data overdetermine the five unknowns, and this redundancy offers us the opportunity to reduce the effective amplitude noise (it also allows a test of Einstein's polarization predictions). In this way, three timing measurements at $\pm 1\%$ and one amplitude measurement with effective error $\pm 3\%$ can be used to locate the source. This suggests that a positional error of $\pm 3^\circ$ is not unreasonable, giving an error

box of $6^\circ \times 6^\circ$. Before discussing how this can be used to identify the source, it is necessary to describe how the arrival time can be measured to 0.5 ms.

Suppose the data are extracted from the noise by convolution with a 'template' waveform (D. Dewey, in preparation) (one way of implementing a matched filter technique). Then the maximum value of the convolution marks the time at which the signal best matches the beginning of the template. This is the 'arrival' of the wave. If the signal-to-noise ratio is 30 then this convolution will have 3% fluctuations. The arrival time can then be determined only to within an error ε , which is the time shift that reduces the noise-free convolution by 3%. I have performed these convolutions for a template spanning 100–200 Hz with a variety of values of $f/\dot{f}$, and I find $\varepsilon \approx 0.5$ ms. (If the signal-to-noise ratio doubles, this value decreases by $\sqrt{2}$.)

The error box is roughly the size of a Schmidt plate. If such events are optically visible, then a search of the error box may identify the galaxy, whose redshift will determine H_0 to a few per cent (limited by the 'random' velocities of galaxies and the distance accuracy). This accuracy improves with the number of events, N , as $N^{1/2}$. If optical identifications are not possible, then the following statistical method, based on galaxy clustering, should still work.

If we accept that H_0 is less than some $H_{\max}$ (say $120 \text{ km s}^{-1} \text{ Mpc}^{-1}$), then the error box can be surveyed for bright galaxies (up to ~ 15 mag) with velocities below $H_{\max}r$, where we consider at first only events with $r < 100$ Mpc. Existing surveys (see ref. 8) show that galaxies with velocities $< 12,000 \text{ km s}^{-1}$ cluster strongly, with ~ 1 cluster per square degree, and that bright galaxies are good tracers of these clusters. In our error box, therefore, the source ought to be in one of ~ 36 clusters. Each cluster redshift gives a candidate value for H_0 . As r is known to 3%, we can divide the range of H_0 into 30 bins. Each observed coalescence event produces one 'correct' H_0 and 35 spurious ones, which are distributed randomly among the 30 bins with probability $(H_0^2/3H_{\max}^3)dH_0$ that a value lies between H_0 and $H_0 + dH_0$. After N events, the bin at $H_0 = 120$ therefore accumulates the largest number of 'randoms', three times the mean number per bin: $3.5N \pm (3.5N)^{1/2}$. In the worst case, if the true H_0 is 120, we will need $N \geq 2(3.5N)^{1/2}$ to see the true values above the fluctuations in the randoms, or $N \geq 14$. If H_0 is really near 60, then we should see a good peak after only three or four events. These values of N will give H_0 to 3% or better. These numbers are only illustrative, as there are many variables which affect them: for example, the typical masses of the coalescing stars, the actual sensitivities achieved by the detectors, and optical effects such as obscuration by the Galaxy. But they show that even the statistical method looks very promising, given a sufficient event rate.

What, then, is the event rate? Estimates⁹ based on the pulsar birth rate and the fact that the binary pulsar is the only compact-object binary system known with a lifetime $< 10^{10}$ yr suggest that there will be ~ 3 events per yr out to 100 Mpc. At this rate, the Hubble constant would be determined in 1–10 yr. But the event rate is highly uncertain, at least by a factor of 10. Therefore it is possible that this method would work only in the long term, some decades after the interferometers begin working. The question of the event rate deserves more attention from astrophysicists.

A small event rate can be offset to some extent by improving the positional accuracy of each event. Obviously, increasing the detectors' sensitivity will reduce the size of the error box. A dramatic improvement can also be achieved by adding a fifth detector to the network. An extra detector in Asia would provide a longer baseline for the timing accuracy. This would improve the position by perhaps a factor of 2–3, and probably speed up the determination of H_0 by a larger factor if the statistical method needs to be used. If the statistical method is used, it will not be helped by including events from further away (events are visible to ~ 0.5 Gpc at moderate SNR). This is because degrading the

SNR enlarges the error box and increases the confusion with randoms.

Finally, how secure is the model? When two $1.4M_\odot$ neutron stars are emitting 100-Hz waves, their separation will be $a \sim 160$ km, or ~ 10 –15 stellar radii. Tidal effects on the orbital period will be negligible: angular momentum transfers are proportional to $(R/a)^5$, where R is the radius of either star. Eardley and Clark¹ considered the tidal mass transfer, which is not important below 200 Hz unless the smaller star has a mass $\leq 0.3M_\odot$. It seems very likely, therefore, that tidal effects will not seriously contaminate the sample. Post-newtonian gravity introduces corrections of $\sim 1\%$ to the orbital period of these systems; moreover, the rate at which they radiate gravitational waves also has corrections of this order from both post-newtonian and octupole contributions. These last have not been accurately calculated, but it is unlikely that they will limit the coherence of the 'template' with the true signal in a way that degrades the accuracy of the timing measurement by much more than the few per cent we have already allowed for. The great attraction of this method is, therefore, the simplicity of the model. Even if the event rate is low, the value of H_0 obtained in this way should, in the long run, be less troubled by systematic errors than that from other methods. All it needs is the continued development of large-scale gravitational wave detectors.

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Detection of hydrogen cyanide emission from the peculiar oxygen-rich evolved star IRC + 10420

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Chemical reaction models do not predict appreciable amounts of hydrogen cyanide (HCN) in oxygen rich [O]>[C] circumstellar envelopes. Recently, two detections of HCN in oxygen-rich envelopes have been reported¹. Here we report the first detection of HCN in the oxygen-rich evolved star IRC + 10420. This star has been classified as an F8 supergiant and is considered by many to be in transition between a red giant star and a planetary nebula. Its circumstellar envelope may be in a more advanced stage of chemical evolution than red giant envelopes.

The observations were made in October 1985 at the $J = 1 \rightarrow 0$ HCN transition frequency of 88,631.8 MHz, using the 12-m telescope of the National Radio Astronomy Observatory. The receiving system consisted of cooled 3-mm dual-polarization receivers and filter spectrometers of resolutions 500 and 1,000 kHz. The full width at half maximum of the antenna beam was 70 arc s at the HCN frequency. Spectral line intensities are

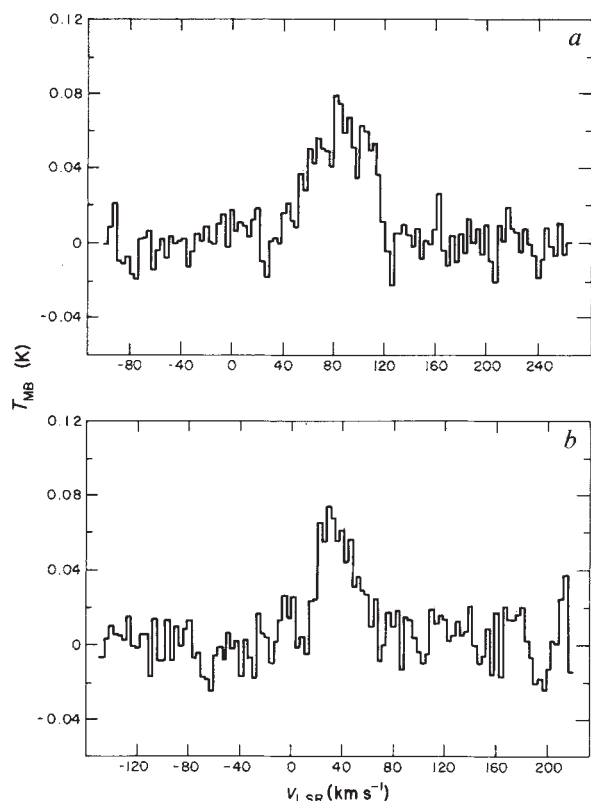


Fig. 1 The $J=1 \rightarrow 0$ HCN emission from IRC+10420 (a) and OH231.8+4.2 (b). The channel resolution for each spectrum is 1 MHz (3.4 km s^{-1}).

given here in units of main-beam brightness temperature, T_{MB} , which is defined as the convolution of the brightness temperature distribution of the source and the gaussian main beam of the antenna. The calibration scale was established by the standard vane chopping technique² and by observations of the planets.

HCN emission in the oxygen-rich stars OH231.8+4.2 and IK Tau has been reported recently by Deguchi and Goldsmith¹. We independently detected HCN in OH231.8+4.2 in observations during September and November 1984. In October 1985, the HCN emission in OH231.8+4.2 was re-observed and the emission from IK Tau was confirmed.

The positive results from the October 1985 observations are summarized in Table 1, which lists each source and its coordinates, the velocity relative to the local standard of rest, V_{LSR} , the velocity width at the base of the line, ΔV_{base} , the peak main-beam brightness temperature T_{MB} (with 1σ uncertainty $\times 100$), and the integrated intensity $\int T_{\text{MB}} dv$. Negative results were found for R Crt, RX Boo, OH17.7-2.0, Vy2-2, and M1-92, all with a 5σ upper limit of $T_{\text{MB}} < 0.1 \text{ K}$, and for IRC+10011, W Hya, WX Ser, VX Sgr, and IRC+10365 with $T_{\text{MB}} < 0.2 \text{ K}$. An HCN line was detected towards OH2.6+0.4, but with $V_{\text{LSR}} = +28 \text{ km s}^{-1}$, so it is most probably line-of-sight interstellar emission; the stellar limit is $< 0.1 \text{ K}$. Figure 1 shows the HCN emission spectra of IRC+10420 and OH231.8+4.2.

IRC+10420 is regarded as a peculiar object for several reasons. Since its discovery³ more than a decade ago, it has been famous as the only known OH/infrared circumstellar maser with a central exciting star of spectral type F8. With the exception of the compact planetary nebula Vy2-2 and possibly the bipolar nebular M1-92, this is the hottest evolved object with OH maser emission^{4,5}—most circumstellar maser sources have central stars with spectral types of M3 or later. The distance to IRC+10420 (3–6 kpc)⁶ is greater than those to other well-known supergiant objects such as VY CMa (1.5 kpc) and NML Cyg (1.8 kpc)⁷. This adds to the measurement difficulty and may partially account for the disagreement about whether the envelope geometry of IRC+10420 is that of a bipolar nebula or a clumpy sphere^{8–10}. The large infrared excess¹¹ of IRC+10420 implies a high dust content. No SiO maser emission has ever been detected from the star¹², which suggests that in the inner envelope, where SiO masers usually reside, the temperature or density of the outflow is different from that of M stars.

The observational data suggest that IRC+10420 is an F supergiant surrounded by a fossil circumstellar shell formed during a prior M giant or supergiant phase of evolution^{13,14}. Provided that the central star reaches ionizing temperatures before the envelope disperses into the interstellar medium, the object will, in this model, become a planetary nebula¹⁵. It may be in a similar stage of evolution to AFGL2688, the famous carbon-rich Egg Nebula, which also has an F supergiant as its central star. AFGL2688 has been long regarded as a proto-planetary nebula^{16,17}.

Based on the presence of OH masers and silicate dust emission, the envelope chemistry of IRC+10420 can be classified as oxygen-rich (OH maser emission has never been reported from a carbon-rich envelope). Other molecules^{14,18,19} detected in the source—SiO, NH_3 and CO—are found in both carbon-rich and oxygen-rich envelopes. Before the detection of HCN in OH231.8+4.2 and IK Tau, HCN was thought to exist only in carbon-rich envelopes. The traditional belief has been that in oxygen-rich environments, all available carbon is locked up in the CO molecule. Deguchi and Goldsmith¹ have suggested that CO in the outer circumstellar envelope is photodissociated by ambient interstellar ultraviolet radiation. The carbon freed by this dissociation is then available for reactions leading to the formation of HCN (ref. 20). This scheme is promising, but requires further consideration, as HCN is itself photodissociated at a rate 100 times faster than CO. In fact, HCN has a smaller angular distribution than many other species in the carbon star IRC+10216, a fact attributed to photodissociation in the outer envelope²¹.

Four classes of circumstellar chemical processes have been identified²²: stellar LTE (local thermodynamic equilibrium) reactions whose products are 'frozen out' as they flow into the envelope, grain depletion, envelope gas-phase radical reactions, and photodissociation in the outer envelope. As IRC+10420 may represent a later evolutionary state of objects such as IK Tau and OH231.8+4.2, its chemical processes, particularly those involving gas-phase reactions and photodissociation, may have proceeded to a more advanced state than in the M stars. Comparisons of abundances among these three different stars may clarify the role and importance of each of the four classes of circumstellar chemical processes.

Table 1 HCN detection in oxygen-rich stars

Source	α (1950)	δ (1950)	T_{MB} (K)	V_{LSR} (km s^{-1})	ΔV_{base} (km s^{-1})	$\int T_{\text{MB}} dv$ (K km s^{-1})
IK Tau*	03 h 50 min 43.7 s	11° 15' 29"	0.07(3)	41	37	1.9
OH231.8+4.2†	07 39 58.9	-14 35 43	0.06(1)	40	51	2.3
IRC+10420	19 24 26.7	11 15 10	0.06(1)	78	78	3.5

* First detected by Deguchi and Goldsmith¹.

† Independently detected by Deguchi and Goldsmith¹.

The addition of IRC+10420 to the two stars reported by Deguchi and Goldsmith¹ suggests that the presence of HCN in oxygen-rich circumstellar envelopes may be relatively common. Although HCN emission is more prevalent in carbon-rich than in oxygen-rich stars, the practice of classifying the circumstellar [C]/[O] ratio according to the existence of HCN emission should be exercised with caution.

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Diffraction pattern simulations of quasiperiodic structures

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The recent experimental and theoretical interest in quasiperiodic structures has been inspired by Shechtman *et al.*'s discovery¹ of precipitates in rapidly cooled Al–Mn, Al–Fe and Al–Cr alloys which exhibit $m\bar{3}5$ icosahedral point symmetry. Subsequent work has shown how diffraction patterns with the same symmetries as those observed experimentally can be generated from model frameworks of linked icosahedra^{2,3} and of three-dimensional Penrose tilings (3DPT)^{4,5}, in which space is filled aperiodically by prolate and oblate rhombohedra with equal sides and angles of $\pm\cos^{-1}(1/\sqrt{5})$ (ref. 6). Here we demonstrate that the computed intensities of the diffracted beams of kinematical electron and X-ray diffraction patterns of quasicrystals are sensitive to the choice of atomic decoration of the underlying 3DPT. Significantly, we have been unable to find a unique atomic decoration of the prolate and oblate rhombohedra which can account fully for the experimental diffraction data. We discuss the implications of this in the light of experimental data on the lack of chemical homogeneity of the icosahedral phases.

Although various models have been proposed, the details of the atomic structure of these icosahedral precipitates are still unclear. Shechtman and Blech², for example, suggested that icosahedra could be linked by either their edges or their vertices and obtained a partial agreement with the available X-ray and electron diffraction data, but without specifying the nature of the atoms or how they might occupy the icosahedra. More

recently, Elser and Henley^{7–9} and Guyot and Audier¹⁰ have succeeded in decomposing related crystalline structures of the icosahedral phases $i\text{-(AlMn)}$ ^{7,8,10} and $i\text{-(AlMgZn)}$ ⁹ into structural elements which might occur in Penrose tilings, with the justification that quasiperiodic structures appear to be the limit of sequences of periodic structures with ever larger unit cells⁷. The atomic shuffles necessary to describe these crystalline structures as periodic packings of the 3DPT rhombohedral units can be likened to those invoked in the crystallography of martensitic transformations, as their magnitudes are much less than nearest-neighbour distances within the structures. Alternative ways of decorating the prolate and oblate rhombohedra have been suggested, using density and packing considerations^{11,12}. Whichever approach is used, the differing local order present in the various models will modulate the diffracted beams in an X-ray or electron diffraction experiment in different ways, allowing a comparison of the predicted and experimental diffraction behaviour.

For each model we investigated, the atoms were assumed to decorate uniquely the two rhombohedra whose vertices define a quasiperiodic 3DPT. Kinematical diffraction patterns were simulated to avoid the effects of dynamical diffraction, which are particularly confusing for $m\bar{3}5$ symmetry, and to facilitate comparison with X-ray powder diffraction patterns, taking account of multiplicity and geometric correction factors in the latter. We compared our simulations with the high-resolution (0.01 nm^{-1}) X-ray data for Al–Mn of Bancel *et al.*¹³, whose experimental results are summarized in Table 1 with the reflection multiplicities. Indices for the reflections are listed using the notation due to Bancel *et al.* and that of Elser¹⁴. The multiplicities of each reflection are determined most easily by comparing the stereographic projection of the icosahedral point group, $m\bar{3}5$, with the electron diffraction patterns from high-symmetry zones, most notably the two-fold axes. The positions of these peaks in the electron diffraction patterns are shown in Fig. 1.

Electron diffraction patterns were simulated for the models summarized in Table 2 in terms of the atomic decorations of the prolate and oblate rhombohedra. The sides of the rhombohedra were taken to be 0.46 nm (the value suggested by Elser¹⁴), except for model j, for which the edge length is $0.46/\tau\text{ nm}$, where τ is the golden number $(\sqrt{5}+1)/2$. The edge length of 0.46 nm can be compared with the Al–Al nearest-neighbour distance of 0.286 nm in crystalline aluminium, and an average Mn–Al nearest-neighbour distance of 0.260 nm in crystalline Al_6Mn (ref. 15). Shorter bond lengths than these have, however, been reported in other Al–Mn crystalline phases such as $\alpha\text{(AlMnSi)}$, for which some Al–Al and Al–Mn bond lengths have been found to be as small as 0.253 nm and 0.227 nm respectively¹⁶.

The models were chosen on the basis of one or more of the following criteria: (1) density and packing considerations (see refs 11, 12); (2) stoichiometry; (3) atomic models suggested elsewhere (see refs 9, 12), where a unique atomic decoration of the rhombohedra could be obtained or approximated.

The stoichiometry of the quasicrystalline phase is particularly interesting; while Bancel *et al.*¹³ and Kimura *et al.*¹⁷ give values of 20–22 atom% for the manganese concentration in melt-spun samples of Al–Mn, other work¹⁸ on rapidly solidified Al–5Fe–7Mn powders has given a value of $>40\text{ atom\%}$ for the transition metal concentration of dispersoids enveloped by cells of solidified aluminium. The X-ray traces of these dispersoids clearly identify them as being quasicrystalline (Fig. 3a of ref. 18). In view of this possible uncertainty in the stoichiometry, some flexibility in the range of manganese concentration was allowed in our models. In interpreting the stoichiometry in terms of the atomic occupancies of the prolate and oblate rhombohedra, note that the ratio of the number of prolate rhombohedra to the number of oblate rhombohedra in an infinite 3DPT is the golden number τ .

To simulate the kinematical electron diffraction patterns, small, approximately spherical regions containing 500–1,000

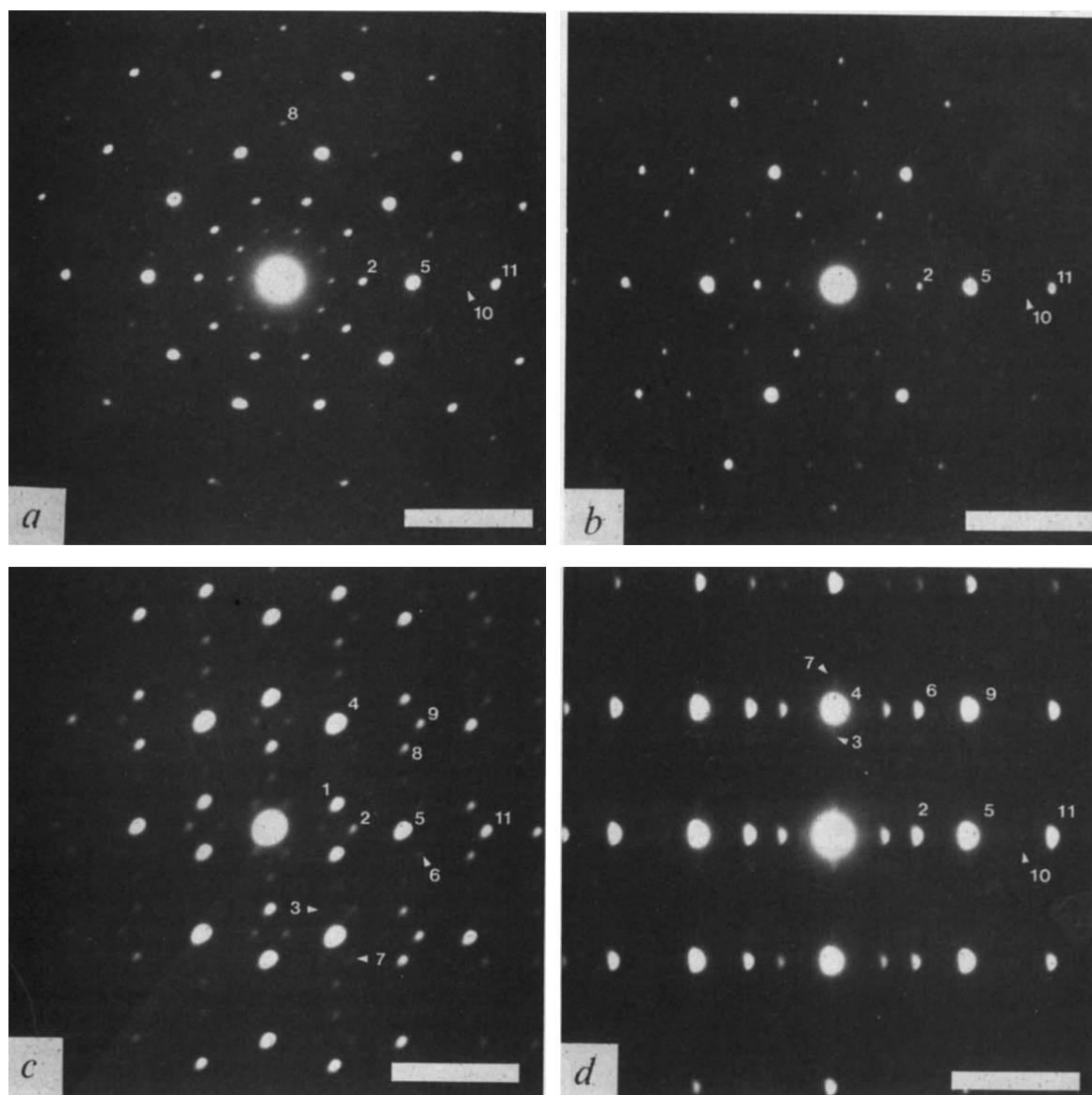


Fig. 1 Experimental electron diffraction patterns from the icosahedral phase in Al-Mn from: *a*, a five-fold axis; *b*, a three-fold axis; *c*, a two-fold axis; and *d*, a mirror axis normal to a five-fold axis. Labelling of the diffraction peaks corresponds to Table 1. Other strong spots in the electron diffraction patterns not appearing in the X-ray data can be explained on the basis of double diffraction. Scale bars, 5 nm^{-1} .

Table 1 Multiplicities, icosahedral indices and effective interplanar spacings, d , for selected prominent diffraction peaks from an icosahedral quasicrystal with rhombohedral sides of 0.46 nm , together with the peak and intrinsic X-ray intensities observed by Bancel *et al.*¹³

Peak label	Interplanar spacing, d (nm)	Index (ref. 14)	Index (ref. 13)	Multiplicity, M	Zone	Relative I_{peak}	Relative $I_{\text{intrinsic}}$	Relative $I_{\text{intrinsic}}/M$
1	0.38591	111000	110001	20	2	22	6.7	8
2	0.33421	111100	111010	30	2, 3, 5, m	8	2.5	2
3	0.28430	111111	311111	12	2, m	1.5	0.7	1.5
4	0.21718	211111	100000	12	2, m	100	48	100
5	0.20655	221001	110000	30	2, 3, 5, m	78	100	83
6	0.18211	222100	111101	60	2, m	—	—	—
7	0.17570	311111	111111	12	2, m	1.5	4.8	1.5
8	0.17570	222101	210001	60	2, 5			
9	0.14967	322101	111000	60	2, m			
10	0.14946	331001	330011	30	2, 3, 5, m	11	25	7
11	0.12766	332002	101000	30	2, 3, 5, m	20	62	52

The intrinsic intensities are obtained from the data of Bancel *et al.*¹³ through the formula $I_{\text{intrinsic}} = I_{\text{peak}} \times \text{peak width} \times \sin \theta \times \sin 2\theta$ for synchrotron radiation (P. A. Heiney, personal communication), where θ is the Bragg angle. Peak widths are taken to be the half-width at half maximum values quoted in Table 1 of ref. 13. For peak 3, their quoted peak width of 0.15 nm^{-1} for the low-resolution measurements was used. The two notations in the literature (refs 13, 14) have been used for the indices of each peak. The symmetries of the electron diffraction patterns in which the diffraction peaks appear are also given.

Table 2 Descriptions of the quasicrystalline models examined in terms of the atomic occupancy of the prolate and oblate rhombohedra whose vertices characterize the 3DPT

Model	Description	No. of Mn and no. of Al Per prolate rhomboheda	Per oblate rhomboheda	Overall Mn concentration (atom %)	Rhombohedral edge length (nm)	Refs
a	Al at all vertices	1 Al	1 Al	—	0.46	14
b	Mn atoms at all vertices of the rhombohedra and Al atoms at all the edge centres and at all the centres of the prolate rhombohedra	1 Mn 4 Al	1 Mn 3 Al	21.65	0.46	11
c	Mn atoms at all vertices of the rhombohedra and Al atoms at all the face centres of the rhombohedra	1 Mn 3 Al	1 Mn 3 Al	25	0.46	22
d	Mn atoms at all vertices of the rhombohedra and Al atoms at all the edge centres of the rhombohedra and along the triad axes of the prolate rhombohedra, dividing the axes in the ratio $\tau:1:\tau$	1 Mn 5 Al	1 Mn 3 Al	19.10	0.46	22
e	Henley and Elser ⁹ model for i-(AlMgZn) modified for i-(AlMn). Prolate and oblate rhombohedra as in model d, but with the maximum possible fraction of rhombic dodecahedra decorated as in Fig. 1c of ref. 9, with Mn atoms at all vertex positions and Al atoms otherwise	1 Mn 5 Al	1 Mn 3 Al	14.64*	0.46	9
f	Mn atoms at the centres of all the prolate rhombohedra and Al atoms at all the vertices and face centres of the rhombohedra	1 Mn 4 Al	4 Al	13.38	0.46	—
g	Mn atoms at centres of all the oblate rhombohedra and Al at all the edge centres. Two atoms, one Al and one Mn, along the triad axes of the prolate rhombohedra, dividing the axes in the ratio $\tau:1:\tau$	1 Mn 4 Al	1 Mn 3 Al	21.65	0.46	—
h	Al atoms at all edge centres of the rhombohedra. Mn atoms at all the centres of all the rhombohedra. Two Al atoms along the triad axes of the prolate rhombohedra, dividing the axes in the ratio $\tau:1:\tau$	1 Mn 5 Al	1 Mn 3 Al	19.10	0.46	—
j	Al atoms at all vertices, except those oblate rhombohedra triad-axis vertices with even parity†	0.75 Al	0.75 Al	—	0.284	—

For 'average atom' models in which no distinction was made between Mn and Al, the average atom was taken to be Al.

* This value arises because the rhombic dodecahedra lose Mn atoms and gain Al atoms on our modification of Elser and Henley's model⁹.

† Omitting Al atoms from oblate rhombohedra triad-axis vertices with even parity (or alternatively odd parity) is necessary to avoid unrealistically short Al-Al nearest-neighbour distances of 0.147 nm. The parity of a particular vertex is determined by whether the integers ($n_1, n_2, n_3, n_4, n_5, n_6$) defining a vertex with respect to the six real-space icosahedral vectors have an odd or even sum.

atoms were generated for each model. For this, a computer program was written to generate sextuplets of integers defining the vertices of prolate and oblate rhombohedra comprising the 3DPT framework. The computational procedure used in the program is described fully elsewhere¹⁹. This 3DPT 'skeleton' was then decorated by atoms for each particular model and the kinematical electron diffraction patterns obtained for a chosen

axis for 500-kV electrons. The structure factor,

$$F(\mathbf{k}) = \sum_{i \text{ atoms}} f_i(\mathbf{k}) \exp(2\pi i \mathbf{k} \cdot \mathbf{r}_i)$$

was calculated for each sampling point $\mathbf{k}$ on a 256 by 256 two-dimensional array normal to the projection direction, summing over each of the i atoms at position vectors $\mathbf{r}_i$ in the region generated. This approach is necessary because we cannot define a unit cell which repeats periodically over all space for a quasicrystalline structure. The atomic scattering factors $f_i(\mathbf{k})$ were estimated using a three-gaussian approximation²⁰. The kinematic intensity $I(\mathbf{k})$ is then simply $F(\mathbf{k})F^*(\mathbf{k})$ and the resultant diffraction pattern intensities were displayed on a logarithmic scale to allow comparison with the experimental data of Bancel *et al.*¹³ Four axes were chosen for these calculations: a five-fold axis $[001000]$, a three-fold axis $[0110\bar{1}0]$, a two-fold axis $[001100]$ and a mirror axis normal to a five-fold axis $[0120\bar{1}0]$ with respect to the icosahedral axes defined by Elser¹⁴, although as Table 1 shows, all the structure-sensitive reflections, the relative intensities of which are displayed in Table 3 for each model, can be seen in the diffraction pattern from the two-fold axis. In Table 1, these four axes are referred to as 5, 3, 2 and m zones, respectively.

Simulated electron diffraction patterns for models a, b and c are shown in Fig. 2. Table 3 and Fig. 2 show that the intensities of the diffraction peaks are model-sensitive, in the same way that in perfect crystalline materials the intensities of the diffraction peaks depend on the atomic motif characterizing the lattice points for a particular Bravais lattice. It is also apparent from Table 3 that for every model examined the relative intensities of the spots fail to give even rough qualitative agreement with

Table 3 Kinematical diffraction intensities for 500-kV electrons for each peak listed in Table 1 for the models described in Table 2, together with the experimental X-ray intensities for 'single crystal' photographs, deduced from the data of ref. 13 and Table 1

Model	1	2	3	4	5	6	7	8	9	10	11
Experimental	8	2	1.5	100	83	—	1.5	7	—	—	52
	w	vw	vw	vs	vs	—	vw	—	w	—	s
a	vs	vs	w	vs	vs	m	w	w	ms	—	s
b	vs	—	w	—	vs	s	w	vw	m	—	s
c	m	s	m	vs	s	s	s	vvw	w	—	m
d	m	—	vvw	m	vs	ms	w	w	w	—	s
e	ms	—	vvw	m	vs	ms	w	w	w	—	s
f	—	s	vw	vs	w	s	s	—	w	—	vvvw
g	—	m	vw	vs	s	m	ms	vvw	m	—	m
h	w	m	vw	vs	m	m	vs	—	—	—	—
j*	—	—	—	m	vs	—	—	—	—	—	vs

s, strong; m, medium; w, weak; v, very.

* Other medium-intensity spots observed at larger effective interplanar spacings than peak no. 11.

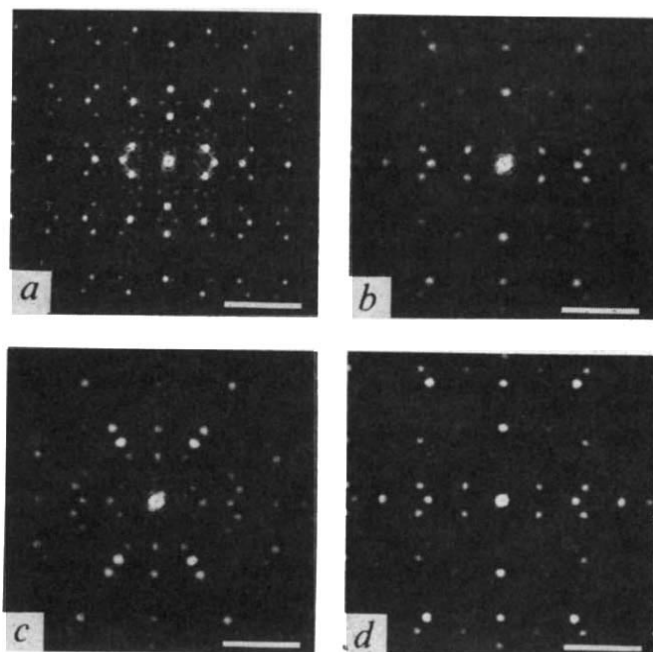


Fig. 2 Simulations of kinematical electron diffraction patterns down a two-fold axis for: *a*, model *a* in Table 2; *b*, model *b* in Table 2; *c*, model *c* in Table 2. *d*, X-ray precession camera diffraction pattern simulation for model *b* in Table 2. Scale bars, 5 nm^{-1} . The intensities are displayed on a logarithmic scale.

the experimental X-ray data. Indeed, the model which appears to offer the best fit is model *a*, an atomistically unrealistic model based solely on vertex occupancy by an 'average' atom. However, in common with all of the models examined, even this model has too strong a peak at an effective interplanar spacing of 0.182 nm . (We use 'effective' to describe the interplanar spacings present in the reciprocal lattice because a reciprocal lattice point from a quasicrystalline material at $1/d$ in reciprocal space does not have a real-space counterpart at d . The one-dimensional analogy of this has been discussed elsewhere²¹). For comparison, the calculations were repeated using X-ray form factors for Al and Mn appropriate for X-ray precession camera photograph simulations instead of atomic scattering amplitudes for 500-kV electrons. The negligible differences between the calculated diffraction patterns for these two radiations (compare Fig. 2*b* and *d*) are attributable to the greater scattering power of electrons and to the steeper slope of the $f_i(\theta)$ versus $\sin(\theta)/\lambda$ curve for electrons in comparison with X-rays. Thus, the X-ray diffraction pattern simulations had less statistical noise in them around 000, but the relative intensities of the reflections for $g \geq 10 \text{ nm}^{-1}$ (outside the range of the interplanar spacings in Table 1) are slightly higher for X-rays than for electrons.

The failure of any of the models in Table 2 to reproduce the experimental X-ray data is significant, as the various positions occupied by the atoms in these models are the only ones which are physically reasonable for unique atomic decorations of prolate and oblate rhombohedra of either 0.46 nm or $0.46/\tau \text{ nm}$ edge length. Furthermore, replacement of Al atoms by Mn atoms and vice versa at selected sites such as the centres of the prolate rhombohedra in model *b*, which can allow a wide range of stoichiometry to be examined for a given model, does not significantly affect the relative diffraction peak intensities for that model. It is therefore not surprising that the model suggested by Elser and Henley⁹ for $i\text{-(AlMgZn)}$, when suitably decorated by Al and Mn atoms (model *e* in Table 2), gives similar diffraction pattern intensities to model *d* (suggested by Hiraga *et al.*²²). It is also apparent from our calculations that none of the models we have considered will account for the X-ray diffraction data

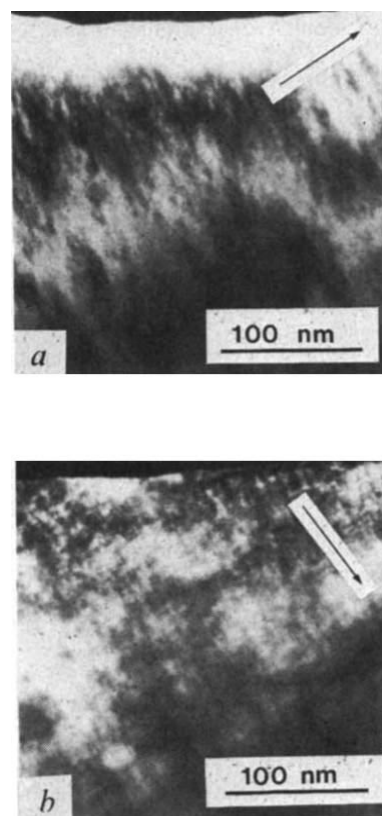


Fig. 3 Dark-field transmission electron microscope images showing cirrus-type contrast: *a*, diffracting vector peak 4 type from Table 1 with the beam direction down an *m* axis; *b*, diffracting vector peak 5 type in Table 1 with the beam direction tilted away from the *m* axis along the systematic defined by peaks 2, 5, 10 and 11, from the same grain as in *a*.

from $i\text{-(PdUSi)}$ ²³, nor can they account for the experimental electron diffraction patterns from other icosahedral phases such as $i\text{-(AlMgZn)}$ ²⁴, $i\text{-(TiVNi)}$ ²⁵ and $i\text{-(AlMgCu)}$ ²⁶, even allowing for the effects of double diffraction. This failure implies either that the icosahedral phases are crystalline after all (see ref. 27), or that the modelling based on atomic decoration of Penrose tiles is either at the wrong scale (that is, the edge lengths of the rhombohedra are wrong by a multiplication factor of τ^n for integer n ; see ref. 14) and/or that a different approach to the modelling is required, perhaps along the lines suggested by Bak²⁸.

Of these two possibilities, we can discount the theory that the icosahedral phase is crystalline, because experimental evidence is incompatible with this²⁹⁻³². Returning therefore to the problem of suitably decorating a quasicrystalline structure with $m35$ point symmetry in reciprocal space, embellishments of the simple unique decoration of prolate and oblate rhombohedra can take many forms. One approach, suggested by Elser and Henley's modelling of $\alpha\text{(AlMnSi)}$ ⁷, is to have two (or more) different decorations of the prolate rhombohedra. A second approach is to allow a 'statistical' occupancy of the two types of rhombohedra, but our preliminary calculations for this possibility do not significantly weaken the intensity of the peaks at the effective interplanar spacing of 0.182 nm . A further possibility, suggested by the work of Stephens and Goldman³, who demonstrated that densely packed assemblies of icosahedra can give sharp diffraction peaks, is to populate their icosahedral units with atoms. However, it is extremely likely that their basic model can also be described on a Penrose tiling basis, with a suitably small edge length and only a very small fraction of the allowed vertices occupied (that is, only those defining local icosahedra).

Evidence for this is provided by the two-dimensional analogy of close-packed assemblies of pentagons—such patterns are also generated by projections down five-fold axes of vertices of the 3DPT (see, for example, Fig. 2a of ref. 19). It is thus unlikely that the scheme suggested by Stephens and Goldman will prove more fruitful than a suitable modelling scheme for the 3DPT.

Experimental work on quasicrystalline materials has yielded several significant and unexpected results, which explain the failure of the atomic occupancy models we have examined and suggest approaches which should be more fruitful, given that a 3DPT framework remains an appropriate basis for a successful model. For example, recent EELS (electron energy loss spectroscopy) work on thin (<10 nm) samples with small (~2 nm) probe sizes has indicated that the icosahedral phase exhibits chemical inhomogeneity on a 3–7 nm scale within a given quasicrystalline grain (D. C. Joy, unpublished data). This observation is broadly consistent with the appearance of dark-field transmission electron microscope images, in that such images appear mottled on this and a coarser scale (Fig. 3). The cirrus nature of this mottling is, however, very different from that seen for either amorphous materials or the spinodal decomposition of a crystalline alloy, because the contrast is a function of the diffracting vector used and the beam direction. Such characteristics are reminiscent of the imaging behaviour of incommensurate lattice distortions, as in the dichalcogenides (see ref. 33); however, taken together with the EELS data, and in the light of Bak's proposal²⁸, this observation implies that some form of generalized chemical wave description superimposed on the 3DPT will be necessary to account for the structure of these materials. Indeed, a careful systematic study of transmission electron dark-field images could be used to suggest appropriate wavelengths and amplitudes in different symmetry directions. A predictive model for why such chemical inhomogeneities might lower the energy of a quasicrystalline phase might in principle involve detailed consideration of how the Fermi surface is affected by the 'Brillouin zone boundaries' in such materials.

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Quantitative bounds on the size spectrum of isotopic heterogeneity within the mantle

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Isotopic heterogeneities within the mantle, as sampled by oceanic volcanism, are reported to exist on all length scales^{1–3}; unfortunately, this fundamental observation has progressed little beyond the qualitative stage. The isotopic systematics of mid-ocean-ridge basalts (MORBs) and ocean-island basalts (OIBs) are now being used to constrain the efficiency of convective mixing^{4,5}; one datum which could constrain convective mixing calculations is the size spectrum of chemical heterogeneity^{6,7}, but unfortunately this datum does not exist. Here I place quantitative bounds on the size spectrum of mantle heterogeneities, using the variations in ⁸⁷Sr/⁸⁶Sr ratios in oceanic basalts over different length scales. The spectrum is consistent with a flat spectrum filtered by magmatism. Such a white-noise spectrum indicates that there are many sources of anomalies at different wavelengths (with about equal magnitude), or that there is a global-scale source of anomalies operating continuously over thousands of millions of years, or some combination of both models.

The isotopic heterogeneity of the mantle and its corresponding size spectrum cannot be measured directly, but it can be indirectly constrained by the isotopic ratios of basalts produced through magmatism. If the length scales of a heterogeneity and of the region of melting are of comparable dimensions, then the spectrum is somewhat distorted; the effects of magmatic filtering will be considered below. It seems likely, however, that at length scales much greater than that of the region of melting, the spectrum should remain undistorted by the sampling process.

The size spectrum will be constrained by using both the ⁸⁷Sr/⁸⁶Sr ratios in basalts sampled along the axes of oceanic ridges and the ⁸⁷Sr/⁸⁶Sr ratios in OIBs over various length scales. The ⁸⁷Sr/⁸⁶Sr ratio was chosen because it is the most extensively measured radiogenic isotope in oceanic basalts; the data were gleaned from an extensive literature search. Moreover, the analysis has been confined entirely to oceanic (MORB and OIB) samples, to ensure that the spectrum has not been subject to continental contamination.

The size spectrum of mantle heterogeneities is constrained by the spatial variations of ⁸⁷Sr/⁸⁶Sr measured in basalts sampled along the axes of mid-ocean ridges. For each ridge segment, the geographical positions of the samples were extrapolated to common linear trends; this yields the data set {⁸⁷Sr/⁸⁶Sr_i, x_i}, where ⁸⁷Sr/⁸⁶Sr_i is the measured value at position x_i. The analysis was set up as an 'overdetermined' problem, and spectral components were extracted by a least-squares analysis. Each datum was given equal weight and overlapping data were averaged together. In order that the misfit with a set of data along a ridge segment be minimized, the coefficients (a₀, a_ks, and b_ks) in the Fourier series

$$\frac{{}^{87}\text{Sr}}{{}^{86}\text{Sr}} = a_0 + \sum_{k=1}^n \left[a_k \sin\left(\frac{2\pi kx}{L}\right) + b_k \cos\left(\frac{2\pi kx}{L}\right) \right]$$

were found. Standard matrix methods of linear regression were employed⁸. *L* is the length of a ridge segment; this formulation explicitly fits a series with period *L*. *n* is the total number of Fourier components found for the particular ridge. The spectral amplitude $\bar{A}_s(k)$ is defined as $(a_k^2 + b_k^2)^{1/2}$. More details on the spectral measurement scheme and data selection and locations can be found elsewhere⁷. The ridge segments studied are summarized in Table 1. For most ridge segments, the data spacing allowed only for $\bar{A}_s$ at *n* = 1 to be found; but, by analysing

Table 1 Ridge segments studied

Ridge	No. of data*	L (km)	n	$\bar{A}_{Sr}$ ($\times 10^5$)	R.m.s. misfit† ($\times 10^5$)	Refs
Atlantic						
Northern Mid-Atlantic Ridge (20–65°N)	103	4,900	8	68.4	16.2	18–25
FAMOUS area	8	17	1	7.5	6.0	26
Near Kane Fracture Zone	4	55	1	9.1	3.0	20
American-Antarctic to South-west Indian (7°W–11°E)	11	620	1	52.9	25.8	27–29
Pacific						
East Pacific Rise						
(24°N–34°S)	45	6,270	1	39.8	11.5	21, 30, 31 22, 23, 32, 33
(17°–23°S)	10	420	1	16.8	7.8	32
(29°–34°S)	5	540	1	3.7	2.7	32
(21°N)	6	7.4	1	7.9	4.5	33
Galapagos spreading centre	34	1,170	2	36.5	8.5	21, 34, 35
Juan de Fuca	31	410	2	16.0	5.9	35, 36
Indian						
South-west Indian (35–70°E)	8	4,280	1	15.5	17.5	22, 37, 38
Central Indian–Carlsberg	15	6,600	1	36.9	12.0	21, 22, 35, 37, 39

* The number of non-overlapping data; data values at the same position were averaged.

† The r.m.s. misfit applies to the entire series up to order n .

Table 2 Variations in ocean-island basalts

Unit	Length scale, L (km)	No. of data	$\langle {}^{87}\text{Sr}/{}^{86}\text{Sr} \rangle$	σ_{Sr} ($\times 10^5$)	Refs
Pacific			Intra-island		
Seamount no. 6, East Pacific Rise	16	5	0.70291	15.5	2
Easter	20	6	0.70320	9.8	40
Guadalupe	20	9	0.70327	5.7	41
Savaii (Samoa)	65	7	0.7059	48	42
Ua Pou (Marquesas)	13	19	0.70470	77.8	43, 44
Nuk Hiva (Marquesas)	22	12	0.70425	51.3	13, 44
Tahiti	40	17	0.7043	47	13, 45
Hawaii	125	17	0.70365	23.2	40, 46
Maui	50	31	0.70345	25.1	47
Oahu	40	21	0.70361	34.1	24, 40, 48
Loihi	17	13	0.70355	9.8	49
Atlantic					
Sao Miguel (Azores)	34	6	0.70456	67.8	28
Terceira (Azores)	26	20	0.70357	11.9	28, 50
St Helena	15	6	0.70290	4.2	40
Tristan da Cunha	11	11	0.70508	4.9	25, 40
Iceland	400	35	0.70315	11.4	19, 25, 51
Bouvet	8	7	0.70315	3.4	25
Ascension	10	8	0.7030	64	25, 52
Indian					
Rodrigues	12	6	0.70374	16.3	53
			Intra-island chain		
Marquesas	260	6	0.70450	70.9	13, 43, 44
Society	300	7	0.70492	76.6	13
Azores	300	6	0.70380	40.5	50
Hawaiian	440	4	0.70357	8.7	24, 40, 46–49
			Intra-ocean		
Atlantic	15,000	10	0.70365	78	
Pacific	6,700	9	0.70374	95	
Indian	3,000	5	0.70391	22	
			Inter-Ocean		
Global	4×10^4	3		13	

well sampled ridge segments of different lengths (4–6,600 km), a wide range of spectral components could be covered.

The size spectrum is also constrained by the variations exhibited by OIBs over different length scales, as summarized in Table 2. Because the geology of oceanic islands does not allow simple linear sets of isotopic ratios versus distance to be made, individual Fourier components cannot be simply extrac-

ted. Therefore, the standard deviation σ_{Sr} between data values (either individual ${}^{87}\text{Sr}/{}^{86}\text{Sr}$ values or spatial averages) was found over an area (for example, over a single island or over an ocean basin). First, the σ_{Sr} between the ${}^{87}\text{Sr}/{}^{86}\text{Sr}$ ratios of individual basalt samples was found for a single island; the samples were generally taken over the entire island and therefore the appropriate length scale is the island's size. Second, the σ_{Sr} between the

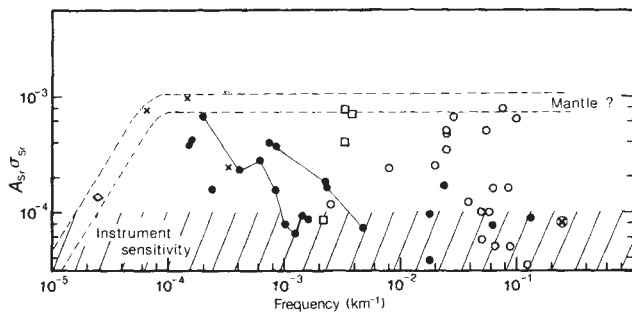


Fig. 1 Size spectrum of $^{87}\text{Sr}/^{86}\text{Sr}$ variations observed in oceanic basalts. Spectra for individual ridge segments where values for $n > 1$ have been determined are connected with solid lines. The lower limit on isotopic heterogeneity is unconstrained. The hatched region labelled 'Instrument sensitivity' brackets the 2σ standard errors of the data used. The band labelled 'Mantle' is the hypothesized true spectrum of the mantle and the diverse amplitudes found at a single frequency are suggested to result from globally variable 'magmatic filtering'. $\bullet$, $\bar{A}_{\text{Sr}}$ for ridges. Other symbols are σ_{Sr} for: $\otimes$, ridges; $\circ$, islands; $\square$, island chains; $\times$, ocean basins; $*$, global.

average $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of individual island members of an island chain was determined. σ_{Sr} was also found for individual oceans by finding the standard deviation between the average $^{87}\text{Sr}/^{86}\text{Sr}$ of islands and chains. Finally, σ_{Sr} was found between three oceans (Atlantic, Indian and Pacific). These σ_{Sr} are plotted as a function of frequency, $(\text{length scale})^{-1}$, in Fig. 1, along with the $\bar{A}_{\text{Sr}}$ found along the axes of ridges. σ_{Sr} and $\bar{A}_{\text{Sr}}$ have been presented on the same plot because variation as measured by the two methods should result in equivalent σ_{Sr} and $\bar{A}_{\text{Sr}}$.

When both ridge axes and island variations are considered, the spectrum (Fig. 1) shows that at any frequency there is a wide range in $\bar{A}_{\text{Sr}}$ and σ_{Sr} . However, no frequency is preferred over any other—there is no hint of a peak at any particular frequency nor is any positive or negative trend evident. Moreover, the maximum amplitude for the different sampled features (single islands, island chains, oceans and ridge axes) was found to be constant within a factor of 1.4 from 10 to 1.5×10^4 km. This quantitatively substantiates the existence of an isotopic heterogeneity on all observable length scales. More fundamentally, however, the spectrum cannot be distinguished from a flat spectrum. Such a spectrum is often called a white-noise spectrum⁹.

Two questions arise from the form of the observed size spectrum: first, what constraints, if any, does a flat spectrum impose on the source of chemical heterogeneity and the mode of convective mixing; and second, what are the likely reasons for the range in observed $\bar{A}_{\text{Sr}}$ and σ_{Sr} at a given frequency.

If the true size spectrum is indeed flat, then some important conclusions follow. Olson *et al.*⁶ have considered the evolution of the spectrum when an anomaly is mixed by a steady cellular flow. Consider an initial anomaly which has a peak at a particular frequency (Fig. 2a, t_0): because the anomaly is stirred by the flow and drawn out into ever finer streaks, this peak in the spectrum is transferred to higher frequencies (for example, t_1 , Fig. 2a). Such a process can be called a 'cascade' phenomenon because individual spectral components are transferred from low to higher frequencies¹⁰. At any time, the spectrum is made up of discrete peaks. However, the spectrum can be white (flat) if new anomalies are continuously injected into and stirred by the convecting system⁶. In this case, the spectrum is essentially a superposition of many peaks over a wide range of frequencies (Fig. 2b). Obviously, a source (or sources) which generates (or generate) equal-amplitude anomalies over a wide range of frequencies would also produce a flat spectrum.

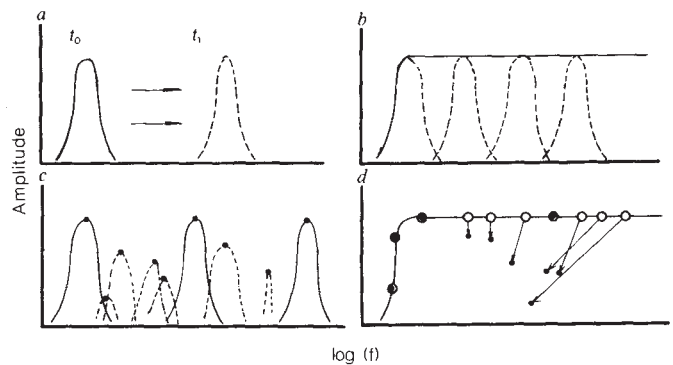


Fig. 2 Hypothetical spectra illustrating the different processes described. *a*, Single anomaly. *b*, Single source in steady state. *c*, Multiple anomalies: solid lines, equal amplitude; dashed lines, smaller amplitudes; $\bullet$, discrete observations unaffected by magmatism. *d*, 'Magmatically filtered' flat spectrum: $\circ$, actual mantle values; arrows denote the effect of magmatism which 'filters' the observed values ($\bullet$).

The wide range in both $\bar{A}_{\text{Sr}}$ and σ_{Sr} at a single frequency suggests that either the actual size spectrum is not white or that magmatism has filtered a flat spectrum. A non-white spectrum with anomalies varying in both frequency and amplitude is illustrated in Fig. 2c; such a distribution of anomalies could satisfy the observed spectrum. This hypothesis is consistent with the possible existence of about five types of mantle heterogeneities, identifiable from Sr, Nd and Pb isotope systematics¹¹. However, because the maximum amplitude is constant over more than three orders of magnitude, there is an indication that there is at least one (semi-) continuous source which produces anomalies of equal amplitude, in addition to sources of anomalies of smaller amplitude.

Magmatism must introduce a certain amount of variation, whatever the form of the actual size spectrum, because σ_{Sr} is inversely proportional to ridge spreading rate¹². Such a relationship suggests that the efficiency of magma mixing (and hence filtering) is globally variable. As illustrated in Fig. 2d, if the magma mixing and spectral length scales are of comparable magnitude, then magmatic filtering will both reduce the amplitude of a heterogeneity and shift the sampled spectral component to lower frequencies. At the longest wavelengths, the length scale of a spectral component is much larger than the scale of magma mixing and the spectrum is not affected by magmatism (Fig. 2d).

Whatever the cause of the range in amplitudes measured at a given frequency, the spectrum requires sources which produce long-wavelength ($\sim 10^4$ km) anomalies. Hart³ has suggested that the longest-wavelength isotopic anomaly (which he finds to be centred in the Indian Ocean) is some sort of stagnant feature, related to inefficient convective mixing. However, the process might be much more dynamic: because other high-frequency spectral components have the same maximum amplitude there is a suggestion that some kind of 'cascade' phenomenon is occurring. Rb-Sr isotopic systematics have demonstrated that the heterogeneity has been in existence for 1–2 Gyr (refs 13, 14); these Rb-Sr systematics are in accord with Pb isotope systematics, where the 1–2 Gyr age signature is less ambiguous¹⁵. This suggests a (semi-)continuous process which produces isotopic anomalies on a scale of $\sim 10^4$ km, which are continuously stirred down to smaller scales. The production and stirring of anomalies occurs with a 1–2 Gyr timescale. Possible sources include: entrainment of material from an adjacent lower layer¹⁶, large-scale delamination of the continents¹⁷, or the breaking up of large convection cells which were previously steady (and isolated from recycled material) for thousands of millions of years⁵.

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Earthquakes recorded stratigraphically on carbonate platforms

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The longest records of earthquake activity¹ may be preserved near the edges of carbonate platforms where reefs have bordered active faults. Because the carbonate sediments build up almost to low tide level, because their character and lithification are very sensitive to deviations from that datum, and because they commonly lithify soon after deposition², strata can precisely record changes in lithospheric flexure³, which in turn record the buildup and release of elastic strain energy during stick-slip faulting. The approximate stratigraphy is predictable for a given history of faulting. Comparison of predictions with observations leads to an explanation for some of the hemicyclic sedimentary sequences common⁴⁻⁶ on carbonate platforms. These records are important for characterizing the seismicity of Atlantic-type continental margins in general. After rifting, faults evidently remain active⁷, but major earthquakes are so rare that their recurrence times cannot be established from direct observations.

Figure 1 shows the margin of a fault-bounded platform during one cycle of stick-slip faulting. Sea level is assumed to rise steadily relative to the platform, so that a continuous and lasting stratigraphic record will accumulate, and to rise slowly enough that sediment can continuously build up to sea level⁸ all across the platform. As long as the fault remains locked, strain accumulates as the platform rises (Fig. 1a) or subsides (Fig. 1d) relative to the adjacent basin. When stress on the fault reaches a critical level, the fault will slip, and stored elastic strain energy will be released in an earthquake (Fig. 1b, e). The fault will then lock, and the cycle will repeat.

In the two examples (Fig. 1), the platform is modelled in two dimensions as an homogeneous, elastic beam (representing the part of the lithosphere that behaves elastically) subjected to sediment loading and to a vertical shear force on the fault at its end (representing the effect of differential subsidence; see Fig. 1 legend). The platform's deflection grows in proportion to the shear force (assumed to be zero immediately after an

earthquake and to increase linearly with subsequent rise in sea level) and dies out as $e^{-x/\alpha} \cos x/\alpha$ with distance x from the fault (Fig. 1a, d). Typical wavelengths $2\pi\alpha$ associated with similar, fault-related deformation are $\sim 100 \text{ km}^9$, or about the same as flexural wavelengths for relatively thin continental lithosphere under carbonate loading. Figure 1d could represent a case in which only the contrast between sediment loading on the platform and water loading in the basin generates the shear force on the fault.

After an earthquake (that is, when the applied force is removed), the platform's surface will be displaced from ambient sea level (Fig. 1b, e). The displacement, as much as a few metres at the platform's margin, will die out approximately as $e^{-x/\beta} \cos x/\beta$ with distance from the fault, where $\beta \approx 0.8\alpha$ (Fig. 1b, e). Displacements that similarly die out with distance from the fault are observed after earthquakes⁹. With time, the topographic relief will die out as sea level continues to rise and as conditions return to normal. In the synthetic stratigraphic sequences (Fig. 1c, f), elastic rebound is assumed to be instantaneous, and bending stresses are assumed to have been entirely relieved through erosion of uplifts to sea level and filling in of basins to that level.

Two hallmarks of repeated stick-slip faulting should be the repetition of serially similar, hemicyclic sedimentary sequences, and the dying out of the cyclicity away from the platform's margin (Fig. 1c, f). Although the great sensitivity of carbonate-secreting organisms and carbonate diagenesis to hydrographic conditions (much less exposure above water level) makes for strong expressions of elastic rebound, it also makes the exact expressions difficult to predict for an actual platform. When the platform's shoulder has been uplifted (Fig. 1b), sediments subsequently deposited on parts exposed above water level should overlie an erosional unconformity (Fig. 1c). When the platform's edge has subsided (Fig. 1e), sediments deposited after the abrupt deepening there should form a shoaling-upward sequence (Fig. 1f, black area). In both cases, uplift at (Fig. 1b) or near (Fig. 1e) the margin could affect sedimentation farther back on the platform for as long as the uplift poses a barrier to exchange of water with the outside. Repeated stick-slip faulting could generate hemicyclic sequences involving abrupt change from normal sedimentation to lagoonal sedimentation of a sort that would depend on climate.

Stick-slip faulting is one of several mechanisms that can generate hemicyclic sequences. Upward and outward growth of mud flats and sediment mounds produces shoaling-upward

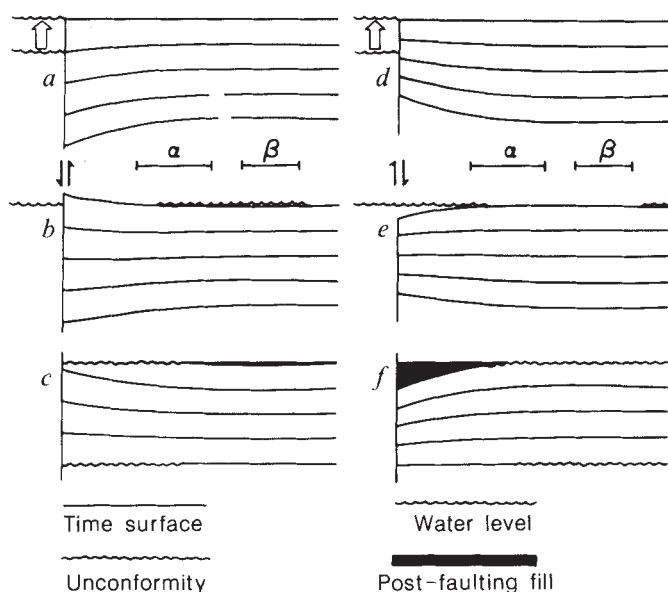


Fig. 1 *a*, Pre-seismic profile across a carbonate platform. The platform is modelled as a homogeneous elastic beam, and accumulation of elastic strain is simulated by a shear force directed vertically downwards along a locked fault at the platform's margin. The magnitude of the force, initially zero, increases linearly with sea level as it rises by the indicated amount. Sediment continuously builds up to sea level as the lithosphere flexes with wavelength $2\pi\alpha$, typically ~ 100 km for earthquake-related deformation⁹. The deflection dies out exponentially, with sinusoidal undulations, away from the margin. The wave's phase depends on the bending moment (zero in this case) applied at the platform's margin. *b*, Profile immediately after the fault slips, assuming instantaneous elastic rebound. The shear force and bending moment on the fault are now zero. The platform's surface is displaced from ambient sea level. The displacement, as much as a few metres at the platform's margin, depending on the stress drop during the earthquake, dies out exponentially with sinusoidal undulations. Neglecting a small component due to the water load, $\beta \approx 0.8\alpha$, reflecting the difference between atmospheric loading and sediment loading. *c*, Synthetic stratigraphic sequence representing one complete cycle of stick-slip faulting. Bending stresses are assumed to be entirely relieved immediately after the earthquake, through erosion of uplifts to sea level and filling in of basins to that level. *d*, Pre-seismic profile as in *a* except that the shear force is opposite. *e*, Corresponding post-seismic profile as in *b*. *f*, Corresponding synthetic sequence as in *c*.

sequences in modern habitats, and cyclical fluctuation in global sea level or regional tectonic subsidence has been proposed for ancient examples (see refs 6 and 10 for reviews). Where evidence permits, the applicability of stick-slip faulting can be tested on several points, as illustrated below. Similar cases in which repeated, serially similar hemicycles are associated with evidence of syn-depositional block faulting at the platform's nearby margin are known from the Mesozoic¹¹, Palaeozoic¹² and Proterozoic^{13,14}. In many more cases, the relationship of cycles to faulting cannot be ascertained because the platform's margin is either unknown or not sufficiently well preserved.

Upper Triassic strata of the Northern Calcareous Alps record a situation like that in Fig. 1*a-c* over ~ 12 Myr (refs 15-19) (Fig. 2). Regional tectonic subsidence dominated relative sea-level change: the small transgressive and regressive fluctuations in global sea level reconstructed for the late Triassic²⁰ are negligible in comparison to the ~ 300 - 500 m that the platform must have subsided relative to final sea level for the 1,000-1,500 m

of penecontemporaneously lithified, shallow water carbonate sediment to have accumulated¹⁶⁻¹⁹.

The L fer cyclothems¹⁶⁻¹⁹ (Fig. 2), serially repeated ~ 300 times in the Dachstein limestone, closely correspond to the hemicyclic sequences expected (Fig. 1*c*) from the platforms tectonic situation (Fig. 2). As expected (and contrary to what would be expected from cyclical sea-level change¹⁷), the cyclicity and the erosional unconformities bounding cyclothems die out within ~ 20 km of the platform's margin, on average, as the Dachstein passes laterally into far back-reef deposits¹⁶⁻¹⁹ (Fig. 2). The apparent width of the platform's uplifted shoulder (Fig. 1*b*), the ~ 20 km width of the Dachstein band (Fig. 2), is in the range expected and in the range observed⁹ for dip-slip earthquakes.

Consistent with expectations, the apparent displacements of the platform's margin relative to ambient sea level are in the few-metres range associated with significant earthquakes. For a given event and location, the displacement will exceed the depth of vadose alteration beneath the erosional unconformity, and will include, in addition, the depth of the lagoon. Only typical values are available: up to ~ 1 m for the depth of alteration¹⁸, and no more than a few metres¹⁸, if that much¹⁷, for the depth of the lagoon.

The distribution of thickness among L fer cyclothems (Fig. 3) is a rough indicator of the frequency of occurrence of earthquakes. Calculated as 300 events during 12 Myr, the average recurrence interval is 40,000 yr. Similar recurrence intervals have been estimated for normal faults in the western United

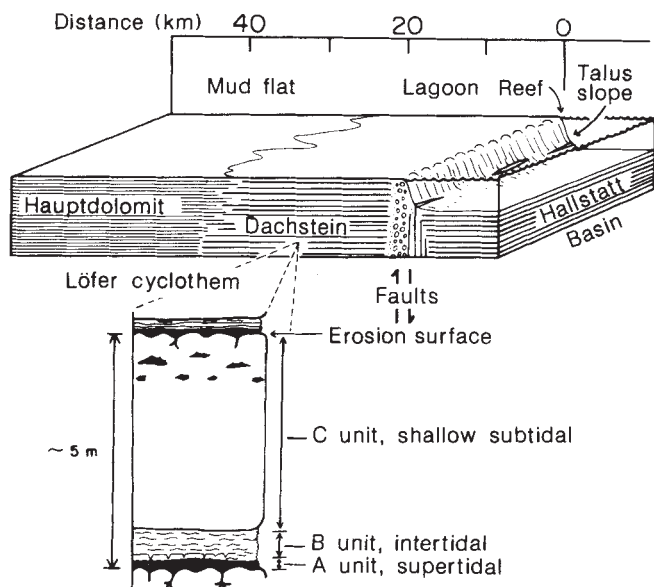


Fig. 2 Profile across the margin of the late Triassic (Norian) carbonate platform of the Northern Calcareous Alps (after ref. 17), and a typical L fer cyclothem (after ref. 18).

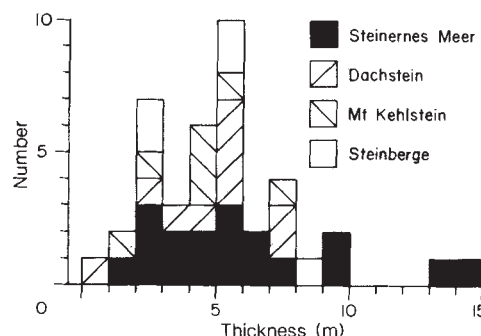


Fig. 3 Histogram of the thicknesses of L fer cyclothems in four sections of the Dachstein limestone. Data on the Mount Kehlstein section are from ref. 17; other data are from ref. 18.

States²¹. Approximately 10–1,000 times longer than typical at plate margins^{1,21}, this mean recurrence time is consistent with the platform's more quiescent tectonic setting. Although many other factors could have contributed to variation, the range in thickness of the cyclothem (Fig. 3) suggests a substantial range in recurrence interval. Megacyclothem of five to seven Löffler cyclothem¹⁸ suggest a longer-term pattern conceivably related to movement on different combinations of fault segments at different times. Studies specifically addressed to seismological questions promise much more precise information from these and other strata.

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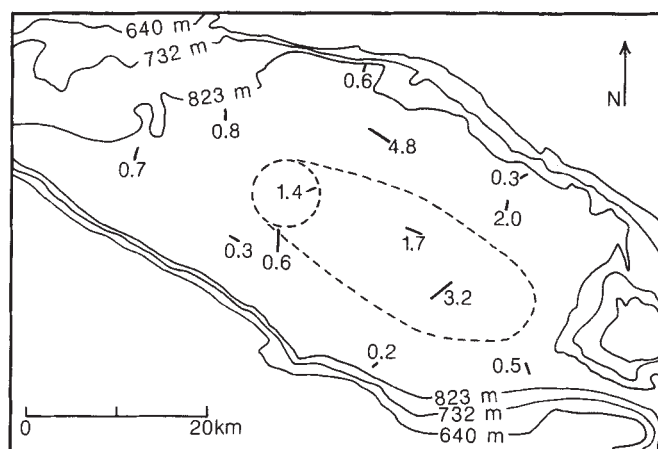


Fig. 1 Bathymetry of Santa Monica Basin. The injection streaks were laid within the dashed circle, which is centred at 33°49' N, 119°00' W. The dashed ellipse shows where SF₆ was found during Leg 2. The short lines show the sampling streaks during Leg 3 and the accompanying numbers give the vertically integrated amount of SF₆ in nmol m⁻².

A deliberate tracer experiment in Santa Monica Basin

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The mixing of heat and chemical constituents across density strata in the ocean plays a major role in ocean circulation and in the response of the climate to thermal and chemical perturbations. We are developing a tracer technique which, in conjunction with fluid dynamical measurements, promises to improve our understanding and our estimates of such diapycnal mixing. In a prototype tracer experiment in Santa Monica Basin, 50 km west of Los Angeles, two tracers, sulphur hexafluoride (SF₆) and perfluorodecalin (PFD), have been injected on an isopycnal surface near the centre of the basin. After 50 days, the tracers had mixed along the isopycnal surface to nearly every part of the basin, although relatively little had penetrated to the basin walls. The diapycnal spreading of the tracer distributions during this first stage of the experiment yielded an apparent eddy diffusivity of $0.33 \pm 0.08 \text{ cm}^2 \text{ s}^{-1}$ at the ambient density gradient of $4.0 \pm 0.5 \times 10^{-9} \text{ g cm}^{-4}$.

Both SF₆ and PFD are extremely safe compounds to handle and are almost certainly harmless in the environment. Under normal conditions they are remarkably inert, both chemically and biologically, and we therefore expect them to be conservative in the oceanic system. Yet they can be detected in amounts

as small as 10⁸ molecules, using gas chromatography with an electron-capture detector.

An enclosed basin was chosen as the site of the experiment, to ensure that the tracers could be found easily. Table 1 and Fig. 1 give bathymetric data for Santa Monica Basin. San Pedro Basin lies to the south-east, beyond a narrow, low saddle, and the two basins together form a single basin with the deepest sill at 737 m, at the southeastern end of San Pedro Basin. Table 1 also lists hydrographic data for the period discussed here, the uncertainty intervals encompassing changes which occurred between sampling legs.

The tracers were injected from R/V *New Horizon* in a series of streaks, each ~2 km long, located in the circle shown in Fig. 1. SF₆, a very insoluble gas, was injected by saturating cooled water in 200-litre drums, lowering the drums to the 5.15 °C surface, and pumping the water out through a heat exchanger at a rate of 2.2 l min⁻¹. As the water was pumped out of a plastic bag lining the drum, new water of the appropriate salinity entered on the other side of the bag to be brought on deck for another cycle of saturation and injection. Fifteen drums of water, each containing ~0.1 mol SF₆ were injected in this way between 29 August and 4 September 1985.

The PFD, a very insoluble liquid, could be injected in higher concentrations, as it had been emulsified into sub-micron droplets before the cruise. A very fine emulsion is required to ensure that the droplets dissolve before they sink beneath the desired density stratum. Four streaks of emulsion, with a total of 4.3 mol PFD, were interspersed with the 15 SF₆ streaks. The use of two tracers provides a useful duplication of the dispersion measurements and allows the evaluation of different tracer compounds and injection methods.

The injection system was kept within 0.01 °C of the 5.15 °C surface by operating the winch in response to the output of a conductivity-temperature-depth probe (CTD) accompanying the injection system. The pump turned off automatically on the relatively rare occasions when the package was outside this temperature range. Temperature is an adequate surrogate for potential density for our purposes, as variations in potential density on the 5.15 °C surface due to salinity and pressure variations are less than ±1 p.p.m.

Sampling was performed from R/V *Robert Gordon Sproul* from 29 August to 16 September and from 18 October to 25 October 1985, with an array of twelve 5-litre Niskin bottles equipped with integrating samplers. These samplers were designed to draw 50 ml of water into glass syringes in 90 min,

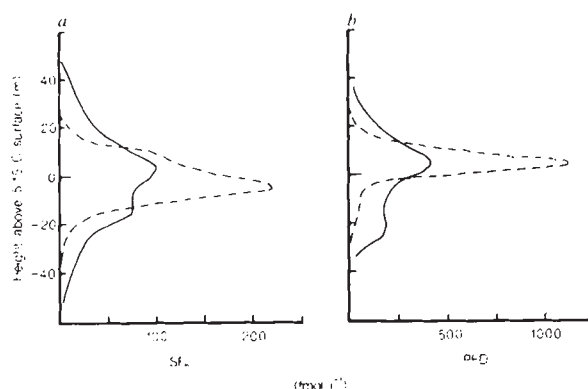


Fig. 2 Vertical spreading of the tracer distributions: average profiles of SF_6 (a) and PFD (b). The background of 1.6 fmol l^{-1} has been subtracted from the SF_6 concentrations. Dashed curve, average of all profiles from Leg 2, ~8 days after injection; solid curve, average of interior profiles from Leg 3, ~51 days after injection. The Leg 3 results have been multiplied by factors of 2.4 and 10.0 for SF_6 and PFD respectively, to make the areas under the curves equal to those under the Leg 2 profiles.

while the array is towed a distance of 1–2 km (Ledwell, Watson, Broecker, Wanninkhof and Sutherland, in preparation). The bottles were spaced at intervals of 5–12 m; with a CTD near the centre of the array. The CTD was held on the 5.15°C surface in the same way as the tracer injection package. The samplers themselves do not remain on unique temperature surfaces, because of the considerable temporal and spatial variation in the fine structure of the temperature profile. The precision of analyses of syringe samples was better than $\pm 3\%$ (1 standard deviation).

During the injection phase, *Sprout* was positioned to drift across individual tracer streaks within a few hours of injection. The second moment of the vertical distribution of tracer about the centres of mass of the eight plumes sampled averaged 41 m^2 . The centres of mass of four of these plumes were $>5 \text{ m}$ below the 5.15°C surface, the deepest being 18 m below. The cause of the large displacement is not known, but subsequent surveys did not reveal peaks so far from the 5.15°C surface, indicating that the displacement was temporary.

After the injection was complete, *Sprout* continued to sample the tracer patch for 13 days with the integrating samplers. During this phase (Leg 2), sampling stations were chosen with a view to determining the drift of the patch as well as the vertical distribution. Figure 1 shows the region (dashed ellipse) in which the tracers were found. It can be inferred from this survey that during the two weeks after injection some of the tracer drifted to the ESE with a speed of at least 1.9 cm s^{-1} . Although it appears that the tracer was headed toward San Pedro Basin, the October survey found no tracer there. Thus, the tracer probably moved with a gyre which was largely confined to Santa Monica Basin. (Current meter data which may reveal the flow pattern and the sources of energy for the basin are just now being gathered and analysed (Alan Bratkovich, personal communication, and Barbara Hickey, personal communication)).

Figure 2a shows the average of the 13 SF_6 profiles obtained during Leg 2 (dashed curve). It is not likely that we missed large parts of the tracer patch, as the product of the integral of this curve and the area enclosed by the dashed curve in Fig. 1 equals the amount of SF_6 injected. Because of the smaller number of injection streaks, the PFD was less horizontally dispersed and more difficult to find. The dashed curve in Fig. 2b shows the average of the five PFD profiles.

During the October cruise (Leg 3), SF_6 was found on every sampling track, and PFD on nearly every track, in Santa Monica Basin. These tracks are shown in Fig. 1, together with the

Table 1 Characteristics of Santa Monica Basin

Area at sill depth ⁵	$1,800 \text{ km}^2$
Volume below sill ⁵	208 km^3
Bottom depth ⁵	938 m
Mean depth of 5.15°C surface	$796 \pm 16 \text{ m}$
Sill depth ⁵ (at SE end of San Pedro Basin)	737 m
Mean characteristics at 5.15°C	
Temperature gradient	$1.8 \pm 0.2 \times 10^{-3}^\circ\text{C m}^{-1}$
Salinity gradient	$-0.19 \pm 0.03 \text{ p.p.m. m}^{-1}$
Potential density gradient	$-0.40 \pm 0.05 \text{ p.p.m. m}^{-1}$
Bouyancy period	$53 \pm 4 \text{ min}$

vertically integrated amount of SF_6 . As nearly as can be estimated from these sparsely spaced stations, the amount of SF_6 found in the basin again balanced the amount injected. However, PFD concentrations were lower than expected from mass balance by about a factor of three, for unknown reasons.

The appearance of relatively small amounts of tracer near the boundaries (Fig. 1) is perhaps due to the constraint that motions toward the walls diminish there. Although the diapycnal dispersion of the tracer was much greater near the boundaries, confirming the expectation of enhanced mixing there, the concentrations were too low to have yet affected the diapycnal distribution of tracer in the interior. (A third survey, completed in February 1986, supports this and other inferences reported here; Ledwell, Watson, Broecker, Wanninkhof and Sutherland, in preparation. Sampling will continue until the signal is lost to determine the residence time for the basin.)

The solid curves in Fig. 2 show the average of ten of the SF_6 profiles (excluding the three closest to the 832 m isobath) and the seven PFD profiles obtained in Leg 3. The SF_6 curve has been multiplied by 2.4 and the PFD curve by 10.0 to normalize the areas to those of the Leg 2 profiles. These factors give estimates of the amount of lateral dilution occurring between surveys. Table 2 documents the growth in the second moments

Table 2 Second moments of average vertical profiles

	Time from injection (s)	SF_6 second moment (m^2)	PFD second moment (m^2)
Leg 2	7.0×10^5	77	46
Leg 3	4.4×10^6	348	239

of the two distributions between Leg 2 and Leg 3. The centres of mass of the distributions remained within 3 m of the 5.15°C surface.

The results for SF_6 should be representative of the whole tracer patch, as we apparently sampled most of the patch, and as there is little variation in shape among the individual profiles from each leg. Furthermore, the large areas and long times involved ensure that we have measured the effect of a number of mixing events in the interior of the basin. The smaller growth in the second moment for PFD may be because the PFD patch, being smaller than the SF_6 patch, was affected by a less representative set of mixing events.

If one interprets the vertical dispersion as being due to fickian diffusion acting on a gaussian profile, with constant diffusivity, one obtains diffusivities of 0.37 and $0.26 \text{ cm}^2 \text{ s}^{-1}$ from the growth of the second moments of the SF_6 and PFD distributions respectively. The difference between the two results gives an estimate of the uncertainty of our summary value, weighted towards the SF_6 result, of $0.33 \text{ cm}^2 \text{ s}^{-1}$. This value may be compared with the value of $0.51 \text{ cm}^2 \text{ s}^{-1}$ which we estimate from the review by Gargett¹ for the open ocean at the same stratification, and with that of $0.96 \text{ cm}^2 \text{ s}^{-1}$ which would be estimated from the equation of Sarmiento *et al.*² based on ^{222}Rn and ^{228}Ra data from the very deep ocean.

Very few experiments have used deliberately injected tracers to measure diapycnal mixing in the deep ocean, and these have been limited in duration to a few days^{3,4}. The present experiment shows that it will soon be feasible to perform ocean-scale tracer experiments lasting many years.

Of course, only a finite number of experiments can be performed in the ocean with a given tracer before they begin to interfere with one another. We realize, and we urge others to realize, that tracers should be used carefully and sparingly in the ocean.

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Aqueous aluminium chemistry response to episodic increases in discharge

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Increased aluminium mobilization and transport from edaphic reservoirs to surface waters have been documented as major effects of atmospheric deposition of mineral acids^{1,2}. High concentrations of hydrogen ions and aqueous aluminium in stream water tend to occur at times of heavy runoff^{3–6}. Some forms of inorganic monomeric aluminium are highly toxic to freshwater biota. It is therefore important to know more about changes in aqueous aluminium speciation and associated chemical parameters during high-discharge episodes. We present here recent data from the Birkenes catchment in southernmost Norway, demonstrating that short-term fluctuations in inorganic aluminium in stream water cannot be explained by assuming equilibrium with a single mineral such as gibbsite ($\text{Al}(\text{OH})_3$). In some conditions $[\text{Al}^{3+}]$ may decrease or remain constant with decreasing pH, thus violating the gibbsite equilibrium assumption. Aluminium in stream water is contrasted with data from soil lysimeters which showed higher pH and concentrations of inorganic aluminium. Lysimeter concentrations were less variable with hydrological conditions. To explain the observed patterns of aluminium fraction concentrations and pH, we postulate changes in flow paths with discharge and/or kinetically controlled dissolution of aluminium.

Birkenes, a 0.4-km² forested catchment with shallow podsolc soils on granitic bedrock, is situated 30 km north of Kristiansand, in an area with atmospheric loading of nearly 7 g $\text{SO}_4^{2-} \text{m}^{-2} \text{yr}^{-1}$ (ref. 7). The catchment is drained by three small second-order

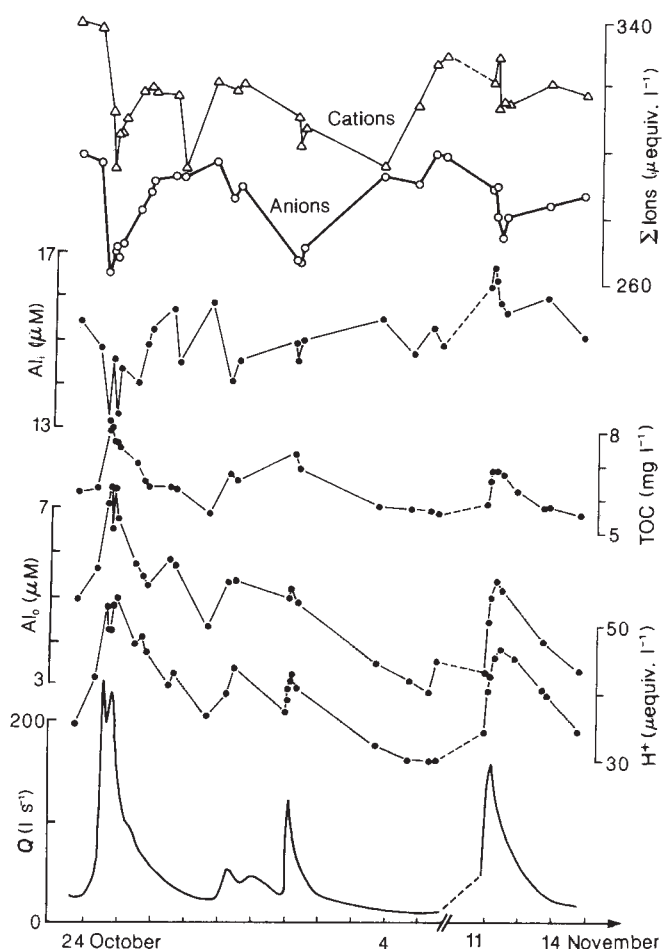


Fig. 1 Stream discharge (Q) and concentrations of selected aqueous species at site BIE 01 during autumn 1984. Ion sums include Ca^{2+} , Mg^{2+} , Na^+ , K^+ , H^+ , Al^{3+} , AlF_2^+ , $\text{Al}(\text{OH})^{2+}$, AlSO_4^+ , SO_4^{2-} , NO_3^- and Cl^- . Concentrations of K^+ were estimated at a constant $8 \mu\text{equiv. l}^{-1}$, based on spring 1985 values and previous autumn measurements from the same site. The observed discrepancy in charge balance is probably mainly due to organic anions, which were not measured.

streams which combine to form a third-order stream 150 m above a V-notch weir (see map inset, Fig. 3). Air and water chemistry and hydrology have been monitored since 1971–72.

Precipitation, surface and soil water samples were collected during three storm events between 24 October and 13 November 1984, and throughout most of the snow-melt period, March–May 1985. Four stream-water sampling stations were established along the main brook and its tributaries (BIE 01, 03, 04, 05; Fig. 3 inset). Autumn sampling was initiated during the latter part of the rainy season when soils were highly saturated. The spring conditions were characterized by a deep snow pack (>1 m) and slow melting during a long cold spring.

Soil water samples were collected from 11 polyethylene tension lysimeters (with a 0.2- μm membrane) and two zero-tension lysimeters, installed in conjunction with the American ALBIOS program⁸. The lysimeters were positioned at varying depths at seven plots, six of which are located along a transect perpendicular to the main brook (Fig. 3 inset). An initial suction of 0.5 bar was applied to each tension lysimeter, which was left for a period of ~8–12 h before sampling. Soil water was collected successfully only in saturated conditions.

Stream and lysimeter samples were transported to an on-site mobile field laboratory and immediately analysed for conductivity, pH and aluminium. Aluminium fractions were separated

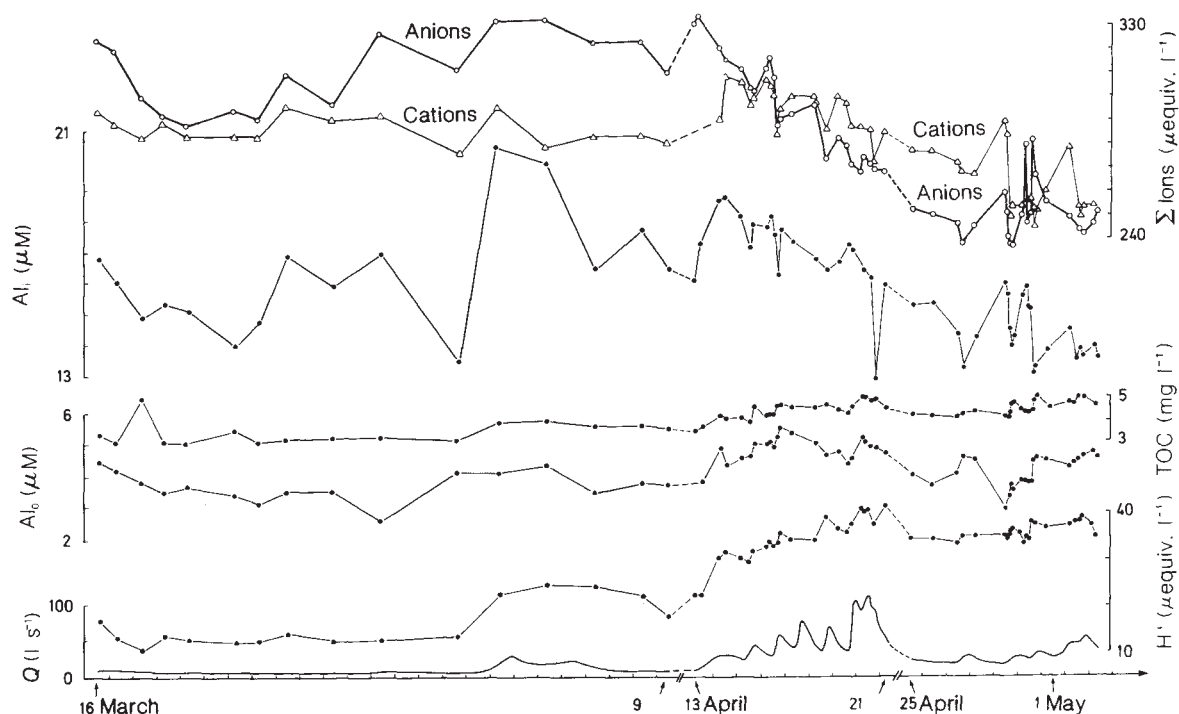


Fig. 2 Stream discharge (Q) and concentrations of selected aqueous species at site BIE 01 during the spring 1985 snow-melt. Individual ions included in ion sums are listed in the Fig. 1 legend. Charge-balance discrepancies are probably mainly due to NH_4^+ and organic anions, neither of which were measured. We expect higher NH_4^+ during early snow-melt, whereas organic anions would be higher during autumn and later snow-melt (with higher TOC).

from unfiltered samples using the Barnes/Driscoll method^{9,10} at ambient temperature, to minimize shifts in speciation during processing. The aluminium fractions, which are operationally defined, include: total acid reactive aluminium (Al_T), non-labile monomeric aluminium (Al_0) (presumably mainly metal-organic complexes) and labile monomeric (mainly inorganic) aluminium (Al_i). Samples were later analysed for major anions, major cations, silica, total fluoride and total organic carbon (TOC). TOC in stream and lysimeter samples was determined indirectly by first measuring ultraviolet absorbance at 254 nm and then constructing calibration curves between TOC and absorbance. Aluminium speciation was calculated using the MINEQL computer program¹¹, which assumes equilibrium of dissolved species. We chose equilibrium constants according to the recommendation of Nordstrom *et al.*¹², which were then temperature-corrected.

Several chemical parameters in samples from the main brook (BIE 01) exhibited interesting relationships with discharge. Non-labile monomeric aluminium (Al_0), total acid reactive aluminium (Al_T), TOC and H^+ were positively correlated with episodic flow. Labile monomeric aluminium (Al_i), total fluoride, silica, NO_3^- , SO_4^{2-} , Cl^- , K^+ (measured only during spring), Na^+ , Mg^{2+} and Ca^{2+} were, in general, inversely correlated with episodic discharge throughout the autumn and the later part of snow-melt (16 April–3 May). Selected species and anion and cation sums are given in Figs 1 and 2. The earlier phases of snow-melt were different in that $[\text{Al}_i]$ increased with $[\text{H}^+]$ and flow. We also observed generally decreasing electrolyte levels as snow-melt proceeded, a trend which was probably related to preferential leaching of pollutants with the first melt water¹³.

Other stream sites (BIE 03, 04, 05), although sampled less intensively, exhibited similar temporal variations. They differed in aluminium concentration, with higher Al_i (including Al^{3+}) in the BIE 03 tributary (Table 1, Fig. 3). The BIE 04 tributary contained the lowest Al_i concentrations. It drains a small bog

area and may be influenced by accumulated organic material, reducing interaction with mineral soils. The upper station on the main brook (BIE 05) had pH levels and aluminium concentrations slightly higher than those at BIE 01 (Table 1).

Lysimeter samples had considerably higher $[\text{Al}_i]$ and pH than were found in stream waters. Soil water chemistry generally showed small variations between the six lysimeter sampling occasions (Table 1). A single suite of samples from four deep lysimeters collected in June 1984, when the stream pH was considerably higher ($\text{pH} = 5.0$), and analysed by the Norwegian Institute for Water Research (unpublished data), were also in close agreement with our measurements.

It has often been assumed that Al^{3+} solubility is controlled by some form of aluminium hydroxide¹⁴ (such as gibbsite), and this assumption has formed an integral part of several mathematical models attempting to describe the effects of acid deposition on aquatic chemistry^{6,15–17}. Although many of the lysimeter samples fall fairly close to the gibbsite line (Fig. 3), our data show that a single gibbsite-type relationship cannot be applied to both soil water and stream water at Birkenes.

There is a large cluster of stream-water sampling points around $\text{pH} = 4.45$, $\text{pAl}^{3+} = 5$, corresponding to $3\text{pH} - \text{pAl}^{3+} = 8.35$. This is between the values for synthetic and natural gibbsite at 25 °C. The actual temperatures were, however, in the range 0–10 °C. The streamwater samples are therefore considerably undersaturated even with respect to synthetic gibbsite (Fig. 3). Only for the stream-water samples from the late winter and early spring is there a positive correlation between pAl^{3+} and pH . But gibbsite equilibrium implies a slope of 3, which is considerably more than is observed.

Recent results from other areas also show that a single value for gibbsite equilibrium cannot always explain stream-water chemistry^{18–20}. Hooper and Shoemaker¹⁸ found a slope close to 1 when pAl^{3+} was plotted against pH for samples from the Hubbard Brook Experimental Forest, New Hampshire.

Table 1 Mean and range of hydrogen ion and labile and non-labile monomeric aluminium concentrations in surface and soil water samples collected at Birkenes during autumn 1984 and spring 1985

Plot*	Lysimeter no.†	Horizon	Hydrogen ion‡ (μM)		Monomeric aluminium‡ (μM)			
			Autumn	Spring	Labile (Al _i)		Non-labile (Al ₀)	
					Autumn	Spring	Autumn	Spring
F	17	B	62 [60-65] (2)	59 [58-60] (3)	ND§	11 [10-14] (3)	ND	10 [9-10] (3)
D	10	O	ND	31 [29-33] (3)	ND	22 [21-22] (3)	ND	5 [5-6] (3)
D	12	B/C	26 [25-28] (2)	ND	30 [29-30] (2)	ND	3 [3-4] (2)	ND
G	18	C	21 [21-21] (2)	26 [23-28] (3)	46 [44-49] (2)	69 [67-70] (3)	3 [3-3] (2)	6 [5-6] (3)
C	Z5	A/B	29 [29] (1)	ND	24 [24] (1)	ND	6 [6] (1)	ND
C	8	B	31 [31] (1)	28 [23-31] (3)	33 [33] (1)	33 [31-35] (3)	6 [6] (1)	7 [6-7] (3)
C	9	B/C	21 [20-22] (3)	17 [15-19] (3)	30 [27-33] (2)	27 [25-30] (2)	3 [3-4] (2)	5 [4-5] (2)
B	Z3	O	37 [37] (1)	ND	23 [23] (1)	ND	3 [3] (1)	ND
B	4	O	ND	32 [32] (1)	ND	16 [16] (1)	ND	3 [3] (1)
B	5	B	29 [28-30] (3)	26 [23-29] (3)	25 [25-25] (3)	22 [21-22] (3)	2 [2-3] (3)	4 [3-4] (3)
B	6	C	28 [27-30] (3)	24 [22-25] (3)	25 [21-28] (3)	24 [24-26] (3)	2 [2-2] (3)	4 [3-4] (3)
A	1	O	24 [22-26] (3)	21 [20-21] (3)	28 [28] (1)	29 [28-30] (3)	3 [3] (1)	4 [4-6] (3)
A	3	C	17 [17-17] (3)	15 [14-16] (3)	30 [29-30] (3)	29 [29-30] (3)	2 [2-2] (3)	4 [3-4] (3)
Site								
BIE 01			40 [30-54] (35)	29 [10-41] (65)	15 [13-17] (29)	16 [13-20] (63)	5 [3-7] (29)	4 [3-5] (65)
BIE 03			38 [31-48] (19)	28 [22-36] (16)	21 [18-22] (13)	21 [15-24] (16)	6 [5-9] (13)	6 [5-7] (16)
BIE 04			44 [32-56] (22)	32 [10-45] (22)	12 [11-19] (15)	13 [9-15] (22)	5 [3-7] (16)	4 [3-5] (22)
BIE 05			37 [29-48] (20)	27 [12-36] (23)	18 [13-23] (15)	18 [17-21] (23)	5 [3-7] (15)	5 [4-6] (23)

* Plots followed a decreasing elevational gradient F-D-G on the west side, and C-B-A on the east side of the catchment (Fig. 3).

† Lysimeter numbers preceded by Z are zero-tension, all others porous tension lysimeters.

‡ Chemical concentration data reported as mean [range] (number of observations).

§ ND, Not determined due to limited sample volume.

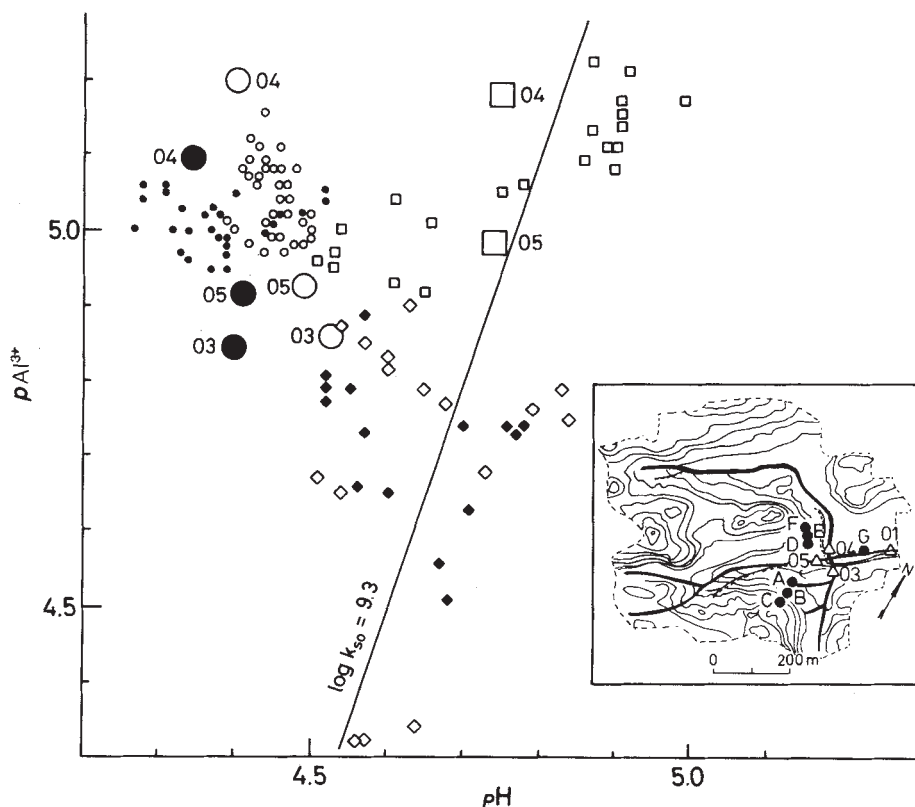


Fig. 3 Relationship between pH and pAl^{3+} for stream and lysimeter samples collected at Birkenes during autumn 1984 (filled symbols) and spring 1985 (open symbols). $\blacklozenge$, $\diamond$, Lysimeter values. $\bullet$, $\square$, $\circ$, Individual samples from BIE 01; $\square$, early spring (16 March-15 April); $\circ$, late spring (16 April-3 May). Average values for other stream sites are plotted similarly but with larger symbols. The gibbsite solubility line ($3pH - pAl^{3+} = 9.3$) is plotted, assuming a solubility product (k_{s0}) corresponding to synthetic gibbsite at 5 °C. Inset, Map of Birkenes catchment showing stream sites BIE 01-05 (triangles) and lysimeter plots A-G (dots).

Nordstrom and Ball²⁰ found a slope ~ 3 for $pH > 4.9$, but a shallow slope for $pH < 4.6$, mainly due to dilution effects. Other suggested relationships for solubility control include equilibrium with $Al(OH)SO_4$ (jurbanite) or $Al_2Si_2O_5(OH)_4$ (kaolinite). General equilibria with these minerals can be excluded at Birkenes. The ion products vary too much, and stream water is undersatur-

ated with respect to jurbanite. $[Al_i]$ follows the same general declining trend during the later part of snow-melt as the total sum of cations, illustrating that other factors are more important than pH in determining $[Al_i]$ in streamwater.

The observed inverse relationship between $[Al_i]$ and $[H^+]$ in stream water during the autumn and later parts of snow-melt

differs markedly from previous studies elsewhere^{5,14,18}. Our data support two possible mechanisms contributing to observed stream-water aluminium chemistry during these highly saturated periods. The decrease in $[Al_i]$ and increase in $[TOC]$, $[Al_o]$ and $[H^+]$ during rain events and melting episodes probably results in part from increased flow through upper organic soil horizons. It is important to realize that equilibrium conditions in organic soil layers could give lower $[Al_i]$ and pH than in mineral horizons because ion-exchange processes rather than mineral dissolution are operating. There is a tendency for $3pH - pAl^{3+}$ to increase with depth within each plot. A potentially important flow path is downward flow through well-drained soils on the steep hill-slopes, followed by upward renewed contact with organic layers near the streams²¹. Saturated conditions can also lead to rain or melt water entering streams so rapidly that equilibrium with soils is not obtained, and runoff is therefore subject to kinetic controls.

The late winter and early spring phases of snow-melt (up to 15 April) merit separate consideration in view of the positive correlation between $[H^+]$ and $[Al^{3+}]$ during this period (Fig. 3). (Very recent results from autumn 1985 indicate a similar concentration pattern for an episode following a long dry period.) Initially $[H^+]$ in stream water is low and $[Al_i]$ moderate (Fig. 2). The substantial increase in $[Al_i]$ with the first minor melting episodes could be due to a piston effect, that is, the first melt water forcing 'old', aluminium-rich soil water into streams²².

A mathematical model simulating major ions in stream water in the Birkenes catchment has previously been developed⁶, and much of the current field work is aimed at improving this model. The model consists of two soil reservoirs (upper organic soils and deeper mineral soils), both incorporating the same gibbsite equilibrium assumption. This was based on earlier measurements of total aqueous aluminium in stream water⁶. Our data show that the aluminium sub-model needs revision. A reasonable modification of the current model structure would be to use different aluminium chemistry in the two soil reservoirs. Future field efforts will include new episode studies with the emphasis on lysimeter work, to model more accurately the soil-water interactions.

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Dental development in *Australopithecus* and early *Homo*

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Human ontogeny requires nearly twice the time as that of living apes¹. This extended period of maturation is usually regarded as a significant evolutionary advance enhancing the importance of learning¹⁻⁵. Mann⁶ suggested that alteration of the timing of growth and development occurred very early in hominid evolution, using evidence based on a human-like pattern of dental development identified in juvenile hominid dentitions from South African cave sites (primarily *Australopithecus robustus* from Swartkrans). He interpreted a human-like pattern to indicate a long human-like schedule of maturation. In contrast, recent study of incremental lines in tooth enamel⁷ suggests short developmental periods for *Australopithecus* and even for early members of the genus *Homo*. Here I report patterns of dental development for *A. afarensis*, *A. africanus*, *A. robustus*, *A. boisei*, *H. habilis* and early *H. erectus*, indicating that *A. robustus* and *A. boisei* ('robust' australopithecines) differ from other hominid species. Most early hominids resemble pongids rather than modern humans in patterns of dental development.

Many new juvenile fossil hominids have been recovered^{8,9} since Mann's original study of australopithecine dental development⁶, and standards of human tooth formation have become more widely known¹⁰⁻¹⁵. Recent publication of a chronological

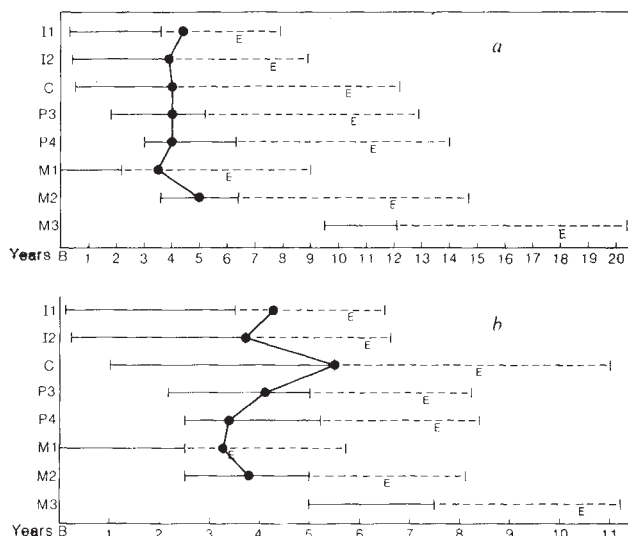


Fig. 1 Mandibular teeth of the Gibraltar 2 Neanderthal child plotted on a, human and b, pongid charts of permanent tooth formation. Charts modified from Dean and Wood¹⁶. Solid lines, crown formation; dashed lines, root formation; E, eruption. The pongid chart is magnified by a factor of 1.8 to equalize periods of pongid and human tooth formation. Gibraltar 2 shows simultaneous events that fit expectations for a human child (that is, plotted points approach a vertical line). The same events are out of synchrony on the pongid chart. Modern human standards are based on longitudinal radiographic studies of mandibular teeth of US caucasian schoolchildren¹⁰ (male-female averages of stages 'crown initialization', 'crown complete' and 'apical closure' shown above differ slightly from Dean and Wood¹⁶). Pongid standards based on cross-sectional study of mandibular and maxillary radiographs of skeletal samples of chimpanzees and gorillas combined with eruption ages drawn from other studies¹⁶. Direct observations are supplemented with radiographs¹⁹ for Gibraltar 2.

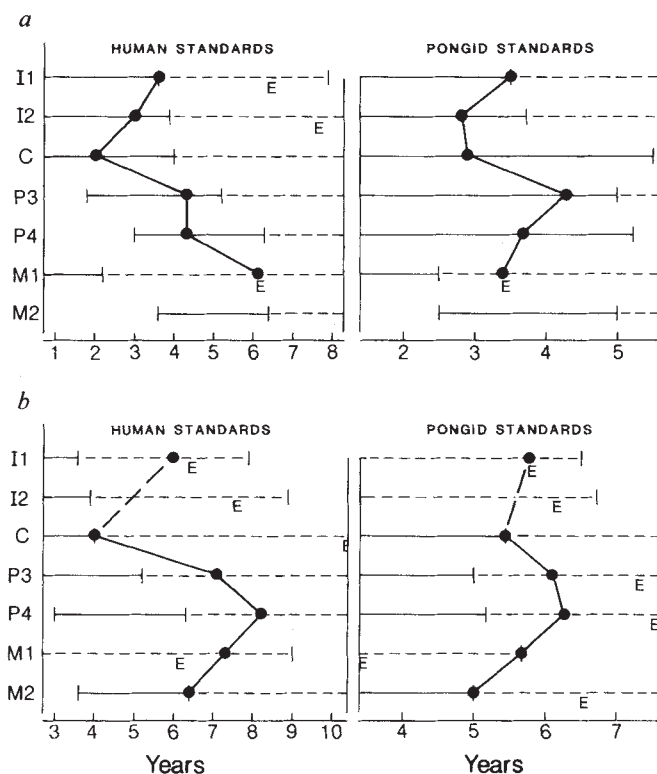


Fig. 2 Maxillary tooth formation of two fossil hominids plotted as humans (left) and as pongids (right), using standards shown in Fig. 1. *a*, STS 24 (Sterkfontein *A. africanus*); *b*, ER 1590 (East Turkana *H. habilis*), both showing such large deviations that it is difficult to assign age at death using human standards. Dashed line indicates missing data for an intervening tooth. Note the delay of anterior teeth relative to M1, characteristic of pongids. Dispersion is lessened when cases are plotted as pongids, particularly for anterior teeth and the first molar.

scale for pongid dental development¹⁶ allows the reassessment of patterns of early hominid dental development with an explicit comparison to living pongids as well as humans. Teeth provide the principal source of developmental information in fossil hominids; there is only one substantial juvenile skeleton of an early hominid with associated skull and dentition identifying it¹⁷.

Dean and Wood¹⁶ presented tooth-crown and root formation of the human and pongid dentition in a summary (Fig. 1). The pongid scale is based on many less data than the human scale and contains some extrapolation, but represents an improvement over information previously available.

Fractional stages of crown development (such as crown one-half complete) can be plotted directly because formation proceeds linearly^{10,11}. Root formation is less simple¹¹, here approximated by allotting the first three-quarters of formation to root elongation and the last one-quarter to apical closure (pongid root formation is plotted directly because standards seem to refer to root elongation¹⁶). Any individual, living or fossil, with developing crowns and roots can be plotted on both human and pongid charts, allowing one to judge which standard provides the best fit by the dispersion of dental ages predicted by individual teeth.

Three methodological points should be noted in Fig. 1: (1) the pongid scale is stretched to make the duration of pongid dental development equal to that of humans, with 10 pongid years equalling 18 human years. This scales dispersion of events in pongids to that of humans in the absence of standard deviations for pongids. (2) the most distinctive feature of pongid dental development, compared with that of humans, is a delay of canines and, to a lesser extent, incisors relative to first molars. (3) charts in Fig. 1 can be applied to both maxillary and mandibular teeth as differences are reportedly small¹².

The example plotted in Fig. 1 is the Gibraltar 2 child, a Neanderthal. Gibraltar 2 shows events that are out of synchrony on a pongid scale (note the canine) but are expected for a human child of four years of age. The fact that a Neanderthal approaches standards based on US caucasian schoolchildren helps illustrate that sex differences¹⁰ and population differences^{15,18} (and judgemental errors of plus or minus one stage¹⁰),

although real, are small on the present scale. Whereas these factors can shift mean values by about one standard deviation, my study is concerned with differences of four to six standard deviation units. Finally, results for the Neanderthal child suggest that one must look farther back in human evolutionary history to find significant developmental differences from living humans.

Formation stages assigned to fossil specimens are based on direct observation of crowns and roots exposed by breakage (radiographs of these heavily mineralized specimens are often poor⁶, and published formation stages¹⁹ are too broad for the present study). Tooth eruption is used as a marker only if slight wear indicates recent eruption before death. Sixteen specimens representing 15 individuals have two more more teeth in diagnostic stages.

Two specimens are plotted on human and pongid charts in Fig. 2. First (*a*), STS 24, a specimen of *A. africanus* from Sterkfontein, is thought to represent a six-year-old child because M1 erupted just before death⁶. All other teeth give far younger ages, ranging as low as two years for the canine. Dispersion is lessened on the pongid chart, where the same developmental events suggest an age of three to four years. Second (*b*), ER1590, a specimen of *H. habilis* from East Turkana, exhibits major deviations from a human pattern: suggested ages range from four years for the canine to more than eight years for the last premolar (P4), a nearly impossible combination for a human child. Standard deviations for events at four years old are about six months in humans, gradually increasing to about one year at ten years of age^{10,14}. If ER 1590 is assigned a mean age of 6.5 years, P4 is $+2.3\sigma$ and C is -5.4σ (if aged on M1, as fossils usually are, C is -6.6σ). By comparison, the pongid scale suggests an age of between five and six years. The magnification of 1.8 for the pongid chart makes these specimens imperfect pongids, but there is an improvement in fit (a factor of 1.8 is the maximum and most conservative that can be used; any smaller factor improves fit to pongid standards).

Dental ages of all specimens are given in Table 1. Numerical measures of fit to standards are based on the dispersion of dental ages interpolated for each tooth.

Table 1 begins with the Neanderthal child and proceeds back

Table 1 Dental ages for juvenile fossil hominids interpolated from human and pongid standards of tooth formation

Taxon		Specimen	Tooth	Dental ages of all teeth (years)												Fit to standards (σ)					
				I1	I2	Human standards			Pongid standards					M1	M2	Human	Pongid†	Best‡			
						C	P3	P4	M1	M2		I1	I2	C	P3	P4	M1	M2			
Homo																					
<i>H. sapiens</i>																					
	Gibraltar 2	md		4.4	3.9	4.0	4.0	4.0	3.5	5.0		4.3	3.7	5.5	4.1	3.4	3.3	3.8	0.47	1.34	H
<i>H. erectus</i>																					
	ER 820*	md		—	7.5	5.5	—	—	—	—		—	6.1	6.9	—	—	—	—	1.41	1.02	P?
	ER 1507	md		—	—	4.0	5.4	6.0	—	6.1		—	—	5.5	5.2	5.0	—	4.8	0.97	0.54	P?
<i>H. habilis</i>																					
	ER 1590	mx		6.0	—	4.0	7.1	8.2	7.3	6.4		5.8	—	5.5	6.1	6.3	5.7	5.0	1.44	0.83	P
Australopithecus																					
<i>A. boisei</i>																					
	OH 30*	mx		—	—	2.9	3.5	4.6	2.2	—		—	—	4.0	3.6	3.9	2.5	—	1.02	1.24	H
	OH 30	md		3.4	—	2.6	—	—	2.2	—		3.3	—	3.7	—	—	2.5	—	0.61	1.10	H
	ER 1171	md		—	—	—	—	6.3	—	6.4		—	—	—	—	5.2	—	5.0	0.07	0.25	H?
	ER 1477	md		3.4	—	2.3	3.5	—	—	—		3.3	—	3.3	3.6	—	—	—	0.67	0.31	P?
	ER 1820	md		3.6	—	—	—	—	2.5	—		3.5	—	—	—	—	2.7	—	0.78	1.02	H
<i>A. robustus</i>																					
	SK 63*	md		—	—	4.3	—	—	6.0	5.4		—	—	5.7	—	—	3.3	4.2	0.86	2.18	H
	SK 839	mx		2.8	3.0	—	—	—	1.7	—		2.7	2.8	—	—	—	1.9	—	0.70	0.89	H
	SK 852	md		3.6	3.6	2.9	—	—	2.5	—		3.5	3.5	4.0	—	—	2.7	—	0.54	0.97	H
<i>A. africanus</i>																					
	STS 24*	mx		3.6	3.0	2.0	4.3	4.3	6.1	—		3.5	2.8	2.9	4.3	3.7	3.4	—	1.39	0.99	P
	STS 24	md		3.6	3.6	—	—	—	6.1	—		3.5	3.5	—	—	—	3.4	—	1.44	0.10	P
	STS 57	mx		—	—	—	4.9	6.0	6.1	4.5		—	—	—	4.8	5.0	3.4	3.3	0.80	1.62	H?
	STW 151*	mx		6.4	5.8	3.8	5.2	—	7.3	6.4		5.8	5.2	5.3	5.0	—	5.7	5.0	1.21	0.72	P
	STW 151	md		6.4	—	4.0	4.9	6.3	7.3	6.4		5.8	—	5.5	4.8	5.2	5.7	5.0	1.20	0.70	P
<i>A. afarensis</i>																					
	LH 2*§	md		3.6	—	3.1	4.0	—	6.1	—		3.5	—	4.4	4.1	—	3.6	—	1.32	0.76	P
	LH 3§	mx		3.9	3.9	2.9	4.3	6.0	6.1	—		3.7	3.7	4.0	4.3	5.0	4.6	—	1.28	0.93	P
	LH 3§	md		—	3.9	3.1	4.3	5.4	6.1	—		—	3.7	4.4	4.3	4.5	4.6	—	1.20	0.64	P
	LH 6	mx		—	5.0	3.8	5.4	—	6.1	—		—	4.6	5.3	5.2	—	4.9	—	0.96	0.57	P

* Abbreviations, mx, maxilla; md, mandible; ER, East Rudolph (= East Turkana); LH, Laetoli hominid; STS, Sterkfontein; STW, Sterkfontein Witwatersrand; SK, Swartkrans; OH, Olduvai hominid.

† Pongid dental ages multiplied by 1.8 to compute σ , scaling human and pongid development to the same length of time. Pongid dental ages shown above are not multiplied.

‡ The standard (H, human, P, pongid) yielding the lower standard deviation of dental age is judged as the better fit. Query denotes equivocal cases lacking data for the crucial contrast between M1 and the anterior teeth (I1, I2, C).

§ First molars of Laetoli hominids 2 and 3 show stages that do not usually coincide in humans: root is one-third on LH 2 (age 3.9) and two-thirds on LH 3 (age 5.6) yet M1s were erupted with some wear (age 6.1). No discrepancy results using the pongid scale.

in time. The clear fit to human standards shown by Gibraltar 2 shifts to better fit by pongid standards, equivocally for early *H. erectus* and more strongly for *H. habilis*. The human pattern emerges as dominant in robust australopithecines, in both cases of *A. robustus* from Swartkrans (SK 839 and 852 count as one as they are the same individual²⁰), and in three of four cases in hyper-robust *A. boisei* from Olduvai and East Turkana. *A. africanus*, a species near the nexus of the two lineages, shows the pongid pattern in two of three cases. The oldest known species, *A. afarensis*, fits pongid standards. Several important specimens are questionable (queries in Table 1) because they lack data for the combination of teeth that usually contributes most to dispersion, M1 versus the anterior teeth. There are insufficient data to resolve the pattern of early *H. erectus*. Cases that disagree with other conspecifics (the sole 'H' in *A. africanus*, the sole 'P' in *A. boisei*) also lack these crucial teeth.

Patterns are clearer when the four specimens lacking data for M1 are discarded. All human charts are superimposed on M1 development in Fig. 3, with remaining teeth scored only in terms of years of advance or delay relative to M1. A clear S-shaped pattern emerges for a 'primitive' group (top) made up of *A. afarensis*, *A. africanus* and *H. habilis*. Incisors and canines are delayed relative to M1 as expected for a pongid. All three species are similar at this scale of resolution. The 'robust' group (Fig. 3, bottom) has diverged from other hominids in dental development. Mann⁶ identified the development pattern of *A. robustus* as human, but the resemblance is probably superficial. Points in Fig. 3 do not fall evenly about the line representing human

development; 11 of 13 teeth are advanced relative to human M1. *A. robustus* and *A. boisei* thus share a unique pattern that is neither human nor pongid.

The strong patterning of *A. afarensis*, *A. africanus* and *H. habilis* dental development relative to human standards does not support the use of a human schedule to assign age at death (it is unclear how one would do so in any case as individual teeth disagree by three and four years). The case for applying a human schedule to *A. robustus* and *A. boisei* is lessened because their dental development appears to be unique and not shared with humans. Mean dental ages assigned using a pongid scale of dental development for LH 2 (3.9 years), STS 24 (3.4), SK 63 (4.4) and ER 820 (6.5) are similar to ages assigned the same specimens by Bromage and Dean⁷ using a completely independent method (compare 3.3, 3.3, 3.2 and 5.3, respectively).

Attribution of a human pattern of dental development to South African fossils⁶ was the sole evidence supporting extended maturation in early hominids, supported by theoretical predictions. Prolonged infant and child dependency appears consistently in theories of early cultural evolution, especially in the origin of a home base, food sharing, division of labour and evolution of mating patterns^{4,21-24}. The assumption that all early hominid species had lengthened periods of development has probably influenced interpretation of the archaeological record, particularly in the level of 'humanness' ascribed to early ancestors^{25,26}. Evidence of dental development presented here, taken together with that of incremental lines⁷, suggests that early hominids had not yet achieved this advanced grade. A human-

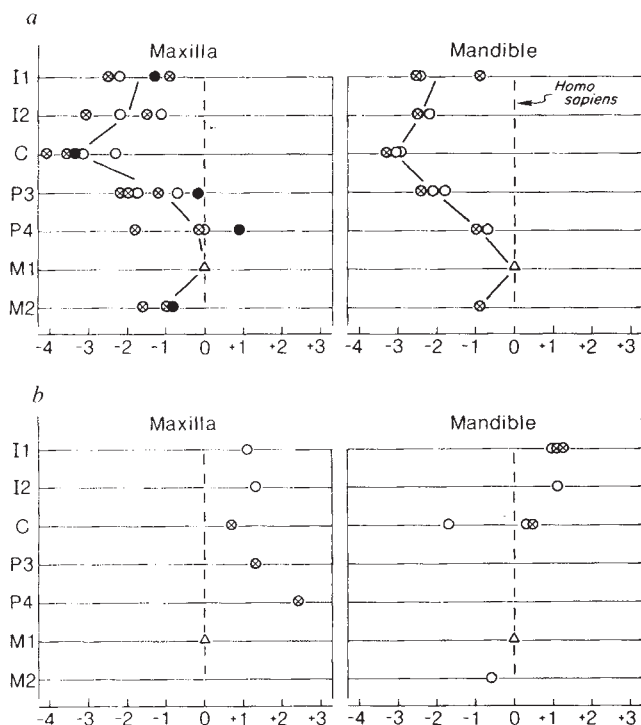


Fig. 3 Pattern of deviation of eleven fossil hominids (cases with data for M1) from human standards of tooth formation. *a*, Primitive group; *b*, robust group. Human charts are superimposed on M1 for the maxilla (left) and mandible (right). Remaining teeth are scored as years of advance (+) or delay (−) relative to M1. Dashed line at zero, position of modern humans. All fossils fall into two groups that do not overlap: *a*, *A. afarensis* (open circles), *A. africanus* (crossed circles) and *H. habilis* (solid circles); *b*, *A. robustus* (open circles) and *A. boisei* (crossed circles). Note that the primitive group shows an S-shaped pattern with most teeth delayed relative to human standards in both maxilla and mandible. In contrast, the robust group tends to show other teeth advanced relative to M1 by human standards, although the data are insufficient to define a clear pattern. The lower canine of SK 63 is an outlier; roots of anterior teeth of SK 63 are slightly damaged¹⁹, which may cause underestimation of age.

like pattern of extended maturation may be a relatively late acquisition in human evolution²⁷. Little is known of ontogeny during the million-year interval encompassed by *H. erectus*. Every juvenile fossil recovered and each new line of evidence is important.

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Suspended clay concentration controlled by filter-feeding zooplankton in a tropical reservoir

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Clay suspended in lake water may be an important factor in limiting primary production by decreasing availability of light^{1–3} and of nutrients^{4,5}. Seasonal changes in the concentration of suspended clay in a lake or a reservoir are usually attributed to the periodicity in hydrological events such as flooding, stratification and mixing^{6–9}. From data presented here it is evident that filter-feeding zooplankton are also important in determining the seasonality of clay abundance. I show that monthly, predator-induced periodicities in the density of zooplankton are synchronized with the periodicities in the rate of decrease in the clay-induced turbidity of the surface waters of a tropical reservoir.

The concentration of suspended clay or silt particles frequently exceeds 1 mg l^{−1} in lakes fed from glaciers and in artificial reservoirs in semi-arid regions. The seasonal fluctuations in clay concentration are in the range of two to three orders of magnitude. The maxima appear suddenly as an effect of floods carrying a large quantity of clay into the lake^{2,6,7}, or as an effect of resuspending clay particles from the sediments during lake overturn^{3,8,9}. The subsequent minima are always more gradual because a long time is needed for fine clay particles to settle. Whereas there is no doubt that any increase in suspended clay concentration must originate from physical processes, I believe it is unlikely that the decreases also result from the purely physical processes of particle sedimentation.

My doubts came from an accidental observation of natural lake water from Cahora Bassa, a large reservoir on the lower Zambezi River, Mozambique, in south eastern Africa (15° 30' S, 30° 20'–32° 40' E). The reservoir (volume 30.5 km³; area 1,902 km²; maximum depth 127 m) is heavily loaded with particles of clays such as montmorillonites, kaolinites and illites (identified by X-ray diffraction), most of which (97%) are 1–2 µm long in the largest linear dimension. I observed the maximum concentration of these particles in the surface waters (10⁹ particles per litre) during and after the lake overturn from May to September, when the turbidity of water determined on a HATCH turbidimeter was >20 JTU (Jackson turbidity units) and Secchi disk transparency <0.9 m (Fig. 1). The lowest concentration (10⁶ particles per litre) was observed at the end of the stratification period from January to March when the turbidity was <3 JTU and transparency >2.5 m despite an increase in the phytoplankton standing crop at that time (Fig. 1). The correlation between the Secchi disk transparency and turbidity

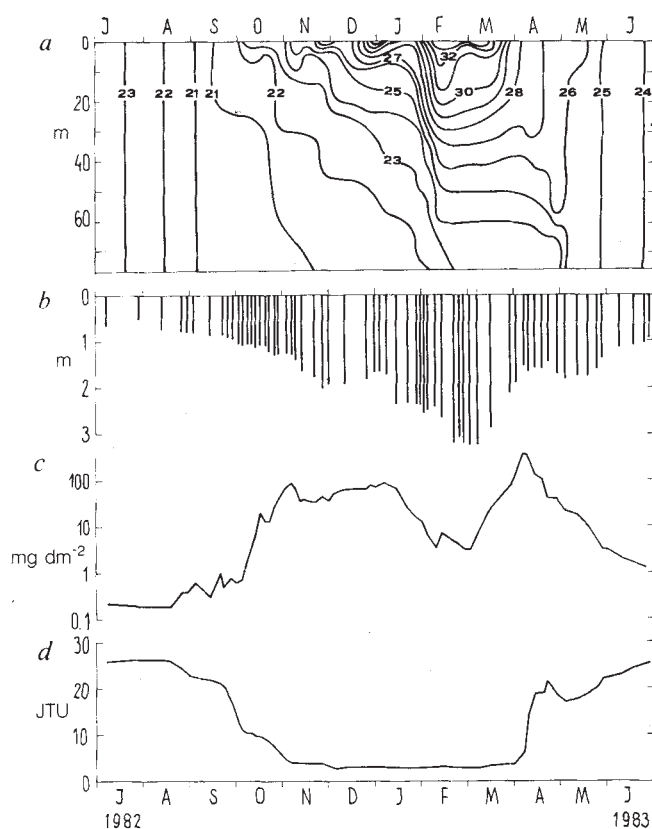


Fig. 1 Seasonal changes in *a*, thermal stratification of Cahora Bassa reservoir; *b*, Secchi disk transparency; *c*, phytoplankton standing crop; *d*, turbidity of the surface (5 m deep) lake water prefiltered on 14- μ m mesh NITEX net. Temperature read with a YSI 5418 thermistor thermometer. Phytoplankton standing crop (integrated volume of all dominant taxa expressed as fresh weight) estimated from counts and cell volume data taken from iodine-preserved samples of unfiltered lake water from the top 10-m stratum. The dominant phytoplankton taxa are flagellated species of *Trachelomonas*, *Eudorina* and *Volvox* from October to February, and diatoms *Synedra* and *Melosira* from March to May^{10,11}

($r = -0.37$) is at the verge of significance ($P = 0.1$) only for data from the period of low phytoplankton (May–October), whereas the correlation between the transparency and the phytoplankton standing crop ($r = -0.59$) is significant at $P = 0.001$ in the period of low clay concentration (November–April).

Neither centrifugation (12,000 r.p.m.) nor sedimentation (in 2-l graduate cylinders) of the lake water is an efficient method for collecting a large sample of clay material for analyses. In June–August, 30 days were necessary for settling down one-half of the suspended clay in 70-cm-high cylinders at 22 °C (decrease in turbidity from 26 to 15 JTU). In October–November the same effect (decrease in turbidity from 6 to 3.5 JTU) was faster (12 days), but during this time much of the clay particles in the lake surface water were observed as 5–30- μ m-large aggregations which sink much more quickly. The settling velocity of the aggregations is 0.08 cm s⁻¹, which is in the range of velocities observed for copepod faecal pellets (0.02–0.11 cm s⁻¹)¹².

I identified these aggregations as cladoceran faeces; the concentration of the material was more than doubled after 24 h when 10–20 cladocerans were added to the 100-cm³ sample of the natural lake water. The faeces each consisted of 10³–10⁵ clay particles. Although less compact than typical copepod faecal pellets and not covered by any peritrophic membrane, they did not break up easily and could be observed easily in preserved samples under an inverted microscope. They became abundant in the lake surface waters in September–October, soon after

high densities of filter-feeding *Diaphanosoma*, *Bosmina*, *Ceriodaphnia* and *Daphnia* (up to 100 cladocerans per l in the top 0–20-m water strata) appeared in the lake that had previously been virtually cladoceran-free (Fig. 2b).

Five independent observations indicate that the filter-feeding activity of cladocerans, resulting in the multiplication of settling velocity of clay material bound into clumps, is the main cause of the decrease in the clay concentration in the lake water.

The first observation is an experimental confirmation that filter-feeding zooplankton (cladocerans more than calanoid copepods) greatly reduce the abundance of clay suspended in the water (Table 1). All the intestines in each of the four cladoceran

Table 1 Size frequency distribution of clay particles in suspension in offshore water on 27 July 1982

Largest linear dimension of particle (μ m)	0–1	1–2	2–3	3–4	4–5
Frequency distribution (parts per thousand)	922	51	19	7	1

species taken from the lake are always filled with clay. No single algal cell nor any other recognizable component was ever encountered in any of the 200 intestines examined. The duration of food passage through the intestine that is found in observations of the guts of animals fed with natural clay mixed with plastic micronized beads, ranges from 15 $\pm$ 5 min for *Bosmina* to 24 $\pm$ 7 min for *Daphnia* at 22 °C.

The second observation is a microscopic estimation of the clay-load volume in cladoceran intestines, combined with the estimation of the duration of food passage. It appears that one adult *Diaphanosoma* of 0.8 mm body length aggregates the clay particles in quantities equal to 3.6 $\times$ 10⁶ μ m³ per day or 1.08 $\times$ 10⁸ μ m³ per month, that is 0.108 mm³ or 0.27 mg of the clay material per month. The respective values estimated for *Bosmina*, *Ceriodaphnia* and *Daphnia* (0.4, 0.7 and 1.1 mm body length, respectively) are 0.054, 0.072 and 0.180 mm³ of clay volume per month. As in July–September the volume of the clay suspended in the surface water amounts to 1 mm³ l⁻¹, I calculated that densities of 5 *Diaphanosoma* or 10 *Bosmina* per litre might aggregate up to 50% of the suspended clay particle within a month.

In the surface waters of Cahora Bassa the cladocerans are most abundant from late August to the beginning of December (Fig. 2b). The population density fluctuates monthly in each species within the range of 0.1 to 60 individuals per litre. The lunar periodicity was induced by predation exerted by a planktivorous freshwater sardine *Limnothrissa miodon* that feeds on zooplankton most efficiently during nights in the phase of the full Moon and soon after, when the full or nearly full Moon rises after sunset, that is, when zooplankton approach the surface during darkness and became suddenly vulnerable in the first light of the rising Moon³. According to estimations based on the food volume in the gut and the duration of food passage, several days before each phase of the full Moon the cladoceran density is high enough to aggregate >1% of the suspended clay daily. At the end of September (during the highest density of *Daphnia*) and at the end of October (during the highest densities of the three remaining species) the density is high enough to aggregate 5% of the clay per day.

The third observation confirming the importance of cladocerans is the sequence of changes in the rate of decrease in the concentration of the clay suspended in the lake surface waters calculated from the turbidity data of Fig. 1. During the decrease in clay concentration, this sequence (Fig. 2a) is highly synchronized with the lunar sequence of changes in cladoceran abundance (Fig. 2b). The correlation between the two parameters ($r = 0.93$) is significant at $P = 0.001$. There is no reason

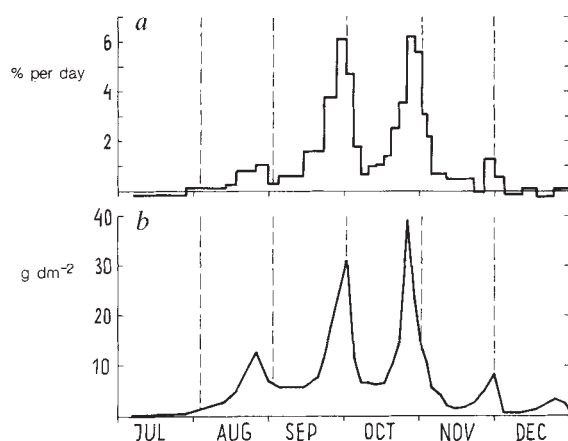


Fig. 2 Lunar periodicity in *a*, the rate of turbidity decrease (per cent turbidity decrease estimated daily from the turbidity data in Fig. 1) and *b*, in the cladoceran standing crop (integrated volume of *Diaphanosoma*, *Bosmina*, *Ceriodaphnia* and *Daphnia*, estimated microscopically from vertical haul samples and expressed as fresh weight) in the period of decreasing concentration of clay suspended in the surface waters of Cahora Bassa reservoir. Vertical broken lines, nights of full Moon.

why a direct effect of the phase of the Moon on the physical factors in the lake should be important. Therefore, it does not seem likely that the periodicity in the rate of clay decrease in that time results from changes in water turbulence and in the intensity of physical aggregation of clay particles.

The fourth observation is on the concentration of clay aggregates in the lake at different dates and depths. In the periods of low cladoceran densities (May–July and December–March) this concentration never exceeds 0.5 cm^{-3} , whereas it is in the range of 9.2 to 67.8 cm^{-3} in September–October. In the latter period higher concentrations are always observed at 60 m rather than at 5 m depth (45.0 and 21.1 cm^{-3} on 22 September; 67.8 and 28.5 cm^{-3} on 19 October, respectively). There are probably two reasons for the higher concentrations in deeper strata: first, the mean depth of each cladoceran population is always well below 5 m deep³; second, cladoceran intestines are more heavily loaded with clay at the time of their descent at dawn than at the time of their ascent at dusk. The second assertion implies that diurnal migration in cladocerans is partly responsible for the fast decreases in the clay concentration in the lake surface waters during the peaks in cladoceran abundance.

The fifth observation is on the dry weight of sediment collected in sedimentation traps suspended in the lake at 20 m depth and exposed for monthly periods: I collected 7 and 4 mg per cm^2 per month in June and July when cladocerans are rare, and 11 , 29 , 27 , 46 , 24 and 13 mg per cm^2 per month in August, September, October, November, December and January, respectively, when cladocerans are abundant. Microevaluation of the fresh samples of the material collected reveals that approximately half of it is composed of aggregations of clay particles.

From April to September, when the phytoplankton standing

crop is low because of high turbidity (Fig. 1), the organic compounds bound to the clay particles seem to be the major food source for filter-feeding cladocerans in the lake, although the organic carbon never constitutes $>2.5\%$ of the dry weight of the clay samples. At maximum clay concentration in the water of $1 \text{ mm}^3 \text{ l}^{-1}$ or 2.6 mg l^{-1} , the concentration of organic carbon bound to clay particles ($0.065 \text{ mg C l}^{-1}$) is only one-third of the threshold concentration of 0.2 mg C l^{-1} , the minimum natural food concentration for egg production in a field population of *Daphnia*¹³. Nevertheless, this concentration is higher than the lowest concentration (0.01 mg C l^{-1}) at which reproduction is observed in *Daphnia* raised under laboratory conditions¹⁴. Both the low food level (low phytoplankton and low organic carbon bound to clay particles) and the high clay concentration prevent high reproduction in cladocerans: from May to August the fecundity is a third of that observed from October to December³. The load of clay in cladoceran intestines increases the gravity of the animals¹⁵ which results in a higher metabolic cost of remaining at desirable depths. This may be why cladocerans are not abundant in this period despite a weakened sardine predation caused by high turbidity^{3,10}. However, once the clay concentration is slightly reduced, higher and higher densities of cladocerans cause its further decrease, which, in turn, allows for a substantial increase in phytoplankton productivity and standing crop.

Zooplankton is often many times more abundant in natural lakes and in reservoirs than it is in Cahora Bassa. Its filter-feeding activity may be responsible for a low summer clay concentration in those stratifying lakes, to which abundant clay materials are carried with the spring floods or are resuspended from sediments during mixing in the spring.

I thank Dr Georg Irion for the X-ray diffraction analyses, and my colleagues at the Institute of Fishery Investigation, Mozambique, for help in the field. The data were collected as part of project FAO/MONAP/GCP/006 (SWE).

Table 2 Effect of zooplankton filter-feeding activity on the concentration of clay particles suspended in lake water

Time of exposure (h)	Control (no animals)	Experimental variants with		
		10 <i>Ceriodaphnia</i>	20 <i>Bosmina</i>	10 <i>Eudiaptomus</i>
		JTU $\times 10^2$ (mean ± 1 s.d.)		
0	1,660 $\pm$ 58			
16	1,630 $\pm$ 60	1,486 $\pm$ 47	1,360 $\pm$ 38	1,552 $\pm$ 76
48	1,602 $\pm$ 45	1,256 $\pm$ 38	1,175 $\pm$ 86	1,490 $\pm$ 96
		Decrease (% control) and significance*		
After 16 h		9†	16†	5
After 48 h		22††	27††	7†

Clay particle concentration was estimated spectrophotometrically as turbidity expressed in JTU $\times 10^2$. Natural lake water without (control) or with (experimental variants) animals was exposed in 100-cm^3 graduate cylinders of 30 cm height kept in the dark at 22°C (28–30 September 1982). For both control and experiments $n = 5$.

* Difference between the untransformed experimental and control sample (Mann–Whitney *U* test) significant at $P = 0.01$ (††) or $P = 0.05$ (†).

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Two polyphosphatidylinositol metabolites control two K^+ currents in a neuronal cell

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Hydrolysis of the membrane phospholipid phosphatidylinositol-4,5-bisphosphate ($\text{PtdIns}(4,5)\text{P}_2$) produces two prospective intracellular messengers: inositol-1,4,5-trisphosphate (InsP_3), which releases Ca^{2+} from intracellular stores¹; and diacylglycerol (DG), which activates protein kinase C^2 . Here we show how the formation of these two substances triggered by one external messenger, bradykinin, leads to the appearance of two different sequential membrane conductance changes in the neurone-like NG108-15 (ref. 3) neuroblastoma-glioma hybrid cell line. In these cells bradykinin rapidly hydrolyses $\text{PtdIns}(4,5)\text{P}_2$ to InsP_3 and $\text{DG}^{4,5}$, raises intracellular Ca^{2+} (refs 4, 6–8) and hyperpolarizes then depolarizes the cell membrane^{4,8,9}. By voltage-clamp recording we show that the hyperpolarization results from the activation pharmacologically-identifiable species of Ca^{2+} -dependent K^+ current. This is also activated by intracellular injections of Ca^{2+} or InsP_3 , so may be attributed to the formation and action of InsP_3 . The subsequent depolarization results primarily from the inhibition of a different, voltage-dependent K^+ current, the M -current¹⁰ that is also inhibited by DG activators. Hence we describe for the first time a dual, time-dependent role for these two intracellular messengers in the control of neuronal signalling by a peptide.

Figure 1 shows the two membrane currents produced by bradykinin in voltage-clamped NG108-15 cells. In each of 63

cells brief local application of bradykinin induced an immediate outward 10–20 s (hyperpolarizing) current, which then gave way to a more sustained inward (depolarizing) current. The former was accompanied by an increase in membrane conductance ($+137 \pm \text{s.e.m. } 56\%$, $n=9$) whereas the conductance was reduced ($-25 \pm \text{s.e.m. } 3\%$, $n=24$) during the inward current.

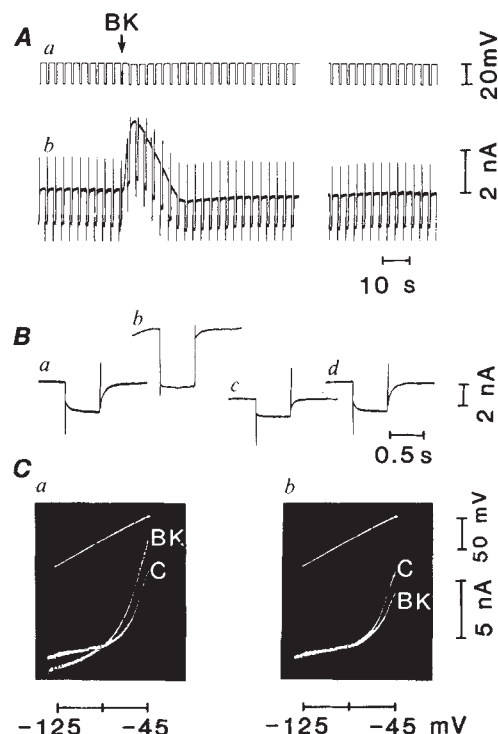
We propose that the initial outward current is a Ca^{2+} -dependent K^+ current generated indirectly through the conversion of $\text{PtdIns}(4,5)\text{P}_2$ to InsP_3 following bradykinin receptor activation and the subsequent release of Ca^{2+} from intracellular stores by InsP_3 . Our principal evidence for this is as follows: first, intracellular iontophoretic injection of Ca^{2+} ions (10 cells) or of InsP_3 (34 cells) (Fig. 2) produced a very similar outward current to that evoked by bradykinin (compare Fig. 1). All three currents reversed at comparable membrane potentials (between -80 and -90 mV), which matches that for the K^+ current activated by a priming Ca^{2+} -current previously described in these cells¹¹. (Figure 3 shows examples of this Ca^{2+} -activated current). Further, the conductances underlying the outward currents produced by bradykinin, Ca^{2+} or InsP_3 were similar in their apparent lack of strong voltage-dependence as judged by subtracting control from test current-voltage curves. This distinguishes the current from some other voltage-sensitive forms of Ca^{2+} -activated K^+ current^{12–14}, and explains why bradykinin can produce a hyperpolarization at rest potential. Equivalent injections of inositol-2-phosphate or inorganic phosphate, which do not release Ca^{2+} from permeabilized cells¹, did not evoke an outward current.

Second, outward currents induced by bradykinin, InsP_2 and Ca^{2+} are also pharmacologically identical in that all three were very sensitive to the Ca^{2+} -dependent K^+ current blockers apamin (0.1 – $0.4 \mu\text{M}$) and D-tubocurarine (0.1 – $0.5 \mu\text{M}$)^{15,16} but were inhibited only by high concentrations (10 mM) of tetraethylammonium, which preferentially blocks voltage-activated K^+ currents in NG108-15 cells¹¹. Figure 3 shows how D-tubocurarine ($200 \mu\text{M}$) reversibly blocked responses to bradykinin and InsP_3 in parallel with the reduction of the slow Ca^{2+} -activated outward current triggered by a priming Ca^{2+} current. This latter current has previously been reported to be blocked by apamin in

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Fig. 1 Changes in membrane currents in voltage-clamped NG108-15 neuroblastoma-glioma hybrid cells induced by applying $2 \mu\text{l}$ of a $10 \mu\text{M}$ solution of bradykinin close to the somal surface. **A**, Continuous time-recording showing an initial outward current followed by a longer inward current. An interval of 80 s separates the two parts of the recovery trace. Downward deflections on the current record are current transients produced by 1 s, 20 mV hyperpolarizing steps applied every 3 s, used to measure input conductance; the current transients are larger during the outward current but smaller during the inward current, implying an increase then decrease in input conductance respectively. **a**, membrane potential; clamp holding potential, -40 mV. **B**, Faster display of current transients in another cell produced by -30 mV, 0.5-s hyperpolarizing steps from a holding potential of -30 mV, sampled, **a**, before adding bradykinin; **b**, during the bradykinin-induced outward current; **c**, during the subsequent inward current; and **d**, after recovery. **C**, Current-voltage (I - V) curves obtained before (**C**) and after (BK) applying bradykinin, the latter being photographed **a**, during the initial outward current; and **b**, during the subsequent inward current. The curves were constructed by applying a 2-s, -90 mV voltage ramp from a holding potential of -45 mV. The recorded voltage was also applied to the oscilloscope X-plates to give a direct display of membrane current (ordinates) against membrane voltage (abscissa). Note that in **a** the two I - V curves intersect at ~ -85 mV, implying that the BK-induced outward current (measured by the difference between the two curves) reverses to an inward current with membrane hyperpolarization; in **b** the two curves do not cross but merge at about -70 mV, so the bradykinin-induced inward current is voltage-sensitive and does not reverse.

Methods. NG108-15 cells were cultured on polyornithine-coated dishes and differentiated using $10 \mu\text{M}$ prostaglandin E_1 and 1 mM theophylline for 2–4 weeks³. For recording, dishes were slowly perfused with fresh Dulbecco's modified Eagle's medium (DMEM) buffered with 20 mM HEPES ($\text{pH } 7.2$) without prostaglandin or theophylline and maintained at about 35°C . The flow rate was $\sim 0.3 \text{ ml min}^{-1}$, giving a bath-exchange time (measured from K^+ equilibration) of 3–5 min. Cells were impaled with microelectrodes filled with 1M K citrate (resistance 20–40 M Ω) and voltage-clamped using a switching amplifier (Axoclamp-2, Axon Instruments) sampling voltage at 5–10 KHz. Bradykinin (Sigma) was dissolved in 150 mM NaCl and applied by pressure close to the cell. The dilution factor, estimated from comparing the effects of drop-applied versus bath-perfused KCl, was 1:3 to 1:8. In all experimental records upward deflections of the current trace indicate outward +ve current and upward deflections of the voltage trace indicate depolarization. Responses to focal bradykinin applications were localized to the cell soma and the first 25–50 μm of any processes. The switching clamp allowed these and other slow somatic responses to be clamped to (normally) within $\geq 90\%$ of the command voltage.



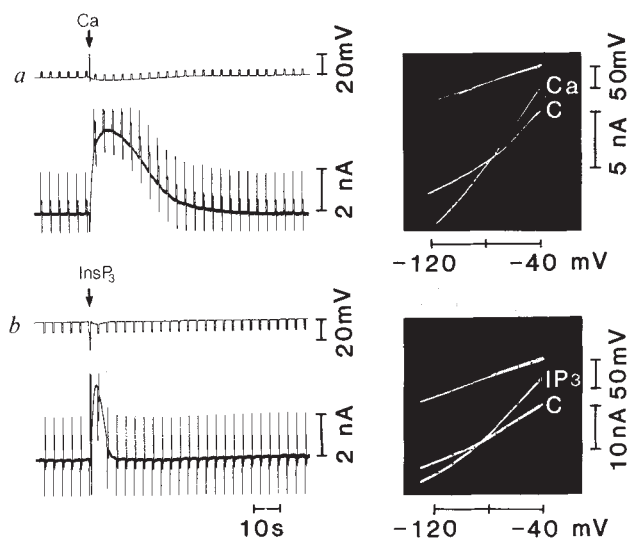


Fig. 2 Outward membrane currents generated by intracellular injections of *a*, Ca^{2+} and *b*, InsP_3 . Left, continuous time-recordings of membrane voltage (upper traces) and membrane current (lower traces) (see Fig. 1A). Right, I-V relations before (C) and during the Ca^{2+} or InsP_3 -evoked outward currents, obtained as described in Fig. 1C but from -40 mV holding potential. To inject Ca^{2+} or InsP_3 the cells were impaled with a second microelectrode filled with 0.5 M CaCl_2 or 0.5 mM InsP_3 (Sigma) in distilled water, and Ca^{2+} or InsP_3 ejected using iontophoretic currents of (nominally) $+20$ nA for 0.25 and -12 nA for 1 s, respectively. Records in *a* are from two different cells, both clamped at -40 mV; those in *b* are from the same cell at different times, clamped at -47 mV (left) and -40 mV (right). CdCl_2 (200 μM) was added to the perfusion fluid for the Ca^{2+} injection tests in *A* (left) to prevent Ca^{2+} influx during the depolarization induced by the Ca^{2+} injection, the injection current being incompletely clamped.

neuroblastoma cells¹⁷ and by both apamin and D-tubocurarine in sympathetic ganglion cells^{18–20}.

Third, both bradykinin and InsP_3 transiently raise intracellular Ca^{2+} in NG108-15 cells; this effect persists in the presence of organic Ca^{2+} channel blockers but is lost in Ca^{2+} -free solution^{6,7}. Correspondingly, we found that the outward currents produced by bradykinin and InsP_3 dissipated after perfusion with a Ca^{2+} -free solution for a few minutes (0 mM Ca^{2+} , 6 mM Mg^{2+}). Because adding Cd^{2+} (0.1 – 0.2 mM) or Co^{2+} (2 – 5 mM) to a normal solution does not have this effect, bradykinin probably does not have to open voltage-gated Ca^{2+} channels to produce the outward current. Instead, we suggest that its action depends upon the availability of an internal Ca^{2+} store which is depleted in Ca^{2+} -free solution.

The secondary bradykinin inward current described above (Fig. 1), results primarily from the inhibition of a voltage-dependent K^+ current closely resembling the M-current originally described in sympathetic ganglion cells¹⁰. Under voltage-clamp this current is characterized by slow inward relaxations during membrane hyperpolarization, reflecting the voltage-induced turn-off of the outward current¹⁰ (Fig. 1B, panel *a*): these relaxations were reduced during the bradykinin-induced inward current (Fig. 1B, panel *c*), signifying that bradykinin itself turns off the current. Other, slower relaxation of as yet ill-defined origin occasionally persist after bradykinin perfusion (see Fig. 4). Because membrane hyperpolarization switches off the M-current, the inward current produced by bradykinin cannot reverse to an outward current with membrane hyperpolarization: instead, the current-voltage curve recorded during the bradykinin inward current simply merged with the control current-voltage curve at potentials of about -70 mV (Fig. 1C, panel *b*).

Unlike the outward current, this bradykinin inward current component is not mediated by the InsP_3 - Ca^{2+} release sequence

because: (1) it was not replicated by either Ca^{2+} or InsP_3 injections; and (2) it persists in Ca^{2+} -free solution. (In several experiments, particularly after large injection currents, we did record an inward current after the outward current following InsP_3 or Ca^{2+} injections. However, this differed from the secondary inward current produced by bradykinin in that it was accompanied by an increased membrane conductance and reversed at potentials between -10 and -20 mV. It might therefore be caused by activation of the non-specific Ca^{2+} -dependent channels previously recorded in neuroblastoma cells²¹, although other possibilities have not been excluded. This matches the secondary depolarization with reduced input resistance seen in unclamped cells after such injections⁸.)

We suggest that the inward current results from the activation of protein kinase C by diacylglycerol (DG), produced together with InsP_3 during bradykinin-induced $\text{PtdIns}(4,5)\text{P}_2$ breakdown⁴, with (presumably) a phosphorylation-induced shutdown of the M-current. Our evidence for this is that application of phorbol-12,13-dibutyrate (0.1 – 1 μM ; 32 cells), which activates protein kinase C², also produced a voltage-dependent inward current and inhibited the M-current (Fig. 4), and thereafter occluded the inward-current response to bradykinin. This was not caused by loss of bradykinin receptor sensitivity because bradykinin still produced an outward current: indeed, the outward current produced by both bradykinin and InsP_3 was enhanced in phorbol dibutyrate solution. The effect of phorbol dibutyrate could be weakly replicated by 1 – 2 μM phorbol-12,13-didecanoate, phorbol-12,13-diacetate and phorbol-12-myristate-13-acetate, and also by 10 μM 1-oleoyl-2-acetylgllycerol, but not by phorbol-13-acetate (2 cells) or 4- α -phorbol (3 cells). The effects of these other phorbol esters, and of oleoyl-2-acetylgllycerol were smaller, less consistent and slower in onset than that of the dibutyrate ester. This, together with the fact that potential change is an indirect and less sensitive index of I_M inhibition than clamp-current measurement²², may explain the previous failure to detect a membrane depolarization following application of phorbol 12-tetradecanoate-13-acetate or 1-oleoyl-2-acetylgllycerol to unclamped cells⁸.

Phorbol esters also inhibit I_M in frog ganglion cells²³ but not in hippocampal pyramidal cells, where they preferentially inhibit a slow Ca^{2+} -activated conductance^{24,25} and a hyperpolarization-activated dendritic chloride current²⁶. In contrast to the hippocampal cells, phorbol dibutyrate did not strongly inhibit either the slow Ca^{2+} -dependent current in NG108-15 cells or the inward Ca current (compare ref. 27). Under our recording conditions NG108-15 cells did not exhibit the phorbol sensitive chloride-current described in hippocampal cells²⁶.

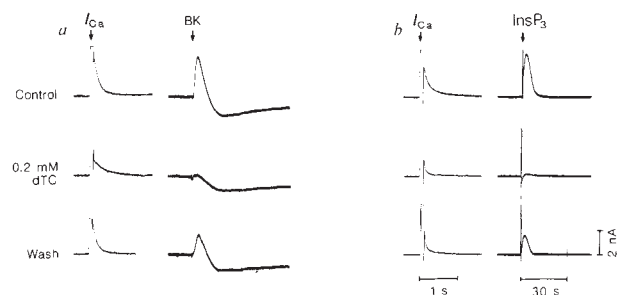


Fig. 3 D-Tubocurarine (dTC, 0.2 mM) inhibits outward currents produced by *a*, bradykinin (BK, 2 μl of a 10 μM solution applied externally); and *b*, intracellular iontophoresis of InsP_3 (-25 nA for 0.5 s). Two cells, clamped at -50 mV. In each experiment drug-induced outward currents were compared with the slow outward current tails following a priming Ca^{2+} current induced by a $+40$ mV, 100 -ms depolarizing command (I_{Ca}). Records from above downwards show currents recorded before adding dTC (upper row, control), 14 (*a*) and 3 (*b*) min after adding dTC to the perfusion fluid (middle row), and 29 (*a*) and 8 (*b*) min after removing dTC (lower row).

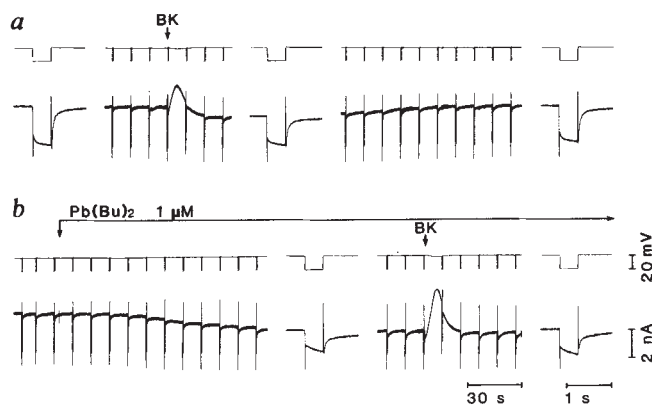
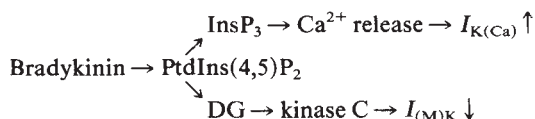


Fig. 4 Phorbol-12,13-dibutyrate ($\text{Pb}(\text{Bu})_2$, $1 \mu\text{M}$, Sigma) produced an inward current and occluded the inward current response to bradykinin (BK, $2 \mu\text{l}$ of $10 \mu\text{M}$). *a*, Bradykinin alone; *b*, $\text{Pb}(\text{Bu})_2$ + bradykinin. *a*, *b*, Continuous recording of membrane voltage (upper records) and membrane current (lower records) in a single cell. The cell was clamped at -41 mV and inward current responses to -20 mV , 0.5 s duration hyperpolarizing commands recorded every 10 s . The recorder was intermittently accelerated $25\times$ to display these current transients. Both bradykinin and $\text{Pb}(\text{Bu})_2$ reduce the total current excursions and reduce the amplitude of the initial faster I_{M} -deactivation component of the inward relaxations during the hyperpolarizing steps, without affecting a superimposed slower relaxation or the fast inward Ca^{2+} -current activated at the end of the hyperpolarizing step. $\text{Pb}(\text{Bu})_2$ was added to the perfusion fluid as a $1:1,000$ dilution of a 1 mM solution in ethanol and applied in dim light; an equivalent dilution of ethanol had no comparable effect.

Our proposed scheme for the action of BK on NG108-15 cells is thus



The temporal relationship between the two membrane conductance changes shown in Fig. 1 suggests that the InsP_3 - Ca^{2+} release sequence is much more transient than the DG kinase C activation sequence. A bifurcating control of two different membrane currents through activation of $\text{PtdIns}(4,5)\text{P}_2$ hydrolysis seems to be involved in some of the responses of oocytes to acetylcholine^{28,29}, although its function is unclear. In NG108-15 cells the effect of the two responses is first to inhibit then accelerate action potential discharges⁴, and thus to modify neuronal signalling.

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The oligodendrocyte-type-2 astrocyte cell lineage is specialized for myelination

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Astrocytes are one of the most numerous cell types in the vertebrate central nervous system (CNS) and yet their functions are largely unknown. In the rat optic nerve there are two distinct types of astrocyte¹: type-1 astrocytes develop from one type of precursor cell², and type-2 astrocytes develop from bipotential, oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells³, that initially give rise to oligodendrocytes (which make myelin in the CNS), and then to type-2 astrocytes⁴. Type-1 astrocytes form the glial limiting membrane at the periphery of the optic nerve⁵ and are probably responsible for glial scar formation following nerve transection⁶. The functions of type-2 astrocytes, which, like oligodendrocytes, are found mainly in tracts of myelinated axons throughout the CNS⁶, are unknown. In this report we provide evidence that processes from type-2 astrocytes contribute to the structure of nodes of Ranvier, suggesting that the O-2A cell lineage is specialized for constructing myelin sheaths and nodes in the mammalian CNS.

Nodes of Ranvier are functionally the most important parts of a myelinated axon. It is here that the myelin sheath is interrupted and current flow is confined; as a result action potentials propagate by jumping from node to node, increasing the speed and efficiency of conduction⁷. In the CNS, astrocytes contribute to the structure of the node by extending fine processes that collectively encircle the exposed axonal plasma membrane⁸. We recently demonstrated that in the adult rat optic nerve the J1 cell adhesion glycoprotein⁹ is concentrated on and around these perinodal astrocyte processes¹⁰. Here we have used a previously described monoclonal antibody called anti-NSP-4 to determine which type of astrocyte extends perinodal processes in rat optic nerve; anti-NSP-4 reacts with several glycoproteins in the mouse CNS¹¹, at least one of which is J1 (M. Schachner and C. Goridis, personal communication). In immunofluorescence double-labelling experiments on semithin frozen sections of adult rat optic nerve rabbit, anti-J1 serum and anti-NSP-4 monoclonal antibody gave superimposable staining patterns, preferentially labelling perinodal processes, with weaker labelling of fine, longitudinally-oriented glial processes (not shown). In double labelling experiments with antibodies against NSP-4 and glial fibrillary acidic protein (GFAP, an intermediate filament protein confined to astrocytes in the rat CNS^{12,13}), all labelled perinodal processes were both NSP-4⁺ and GFAP⁺ (Fig. 1). This pattern of labelling suggests that the NSP-4 antigen is concentrated on and around perinodal astrocyte processes in rat optic nerve, as we showed previously for the J1 glycoprotein¹⁰.

To examine the cell-type distribution of NSP-4 antigen, we cultured optic nerve cells from embryonic day 17 (E17), postnatal day 1 (P1), P7 or P15 rats for 2 or 3 days in 10% fetal calf serum (FCS), conditions in which most O-2A progenitor cells develop into type-2 astrocytes³. When such cultures were

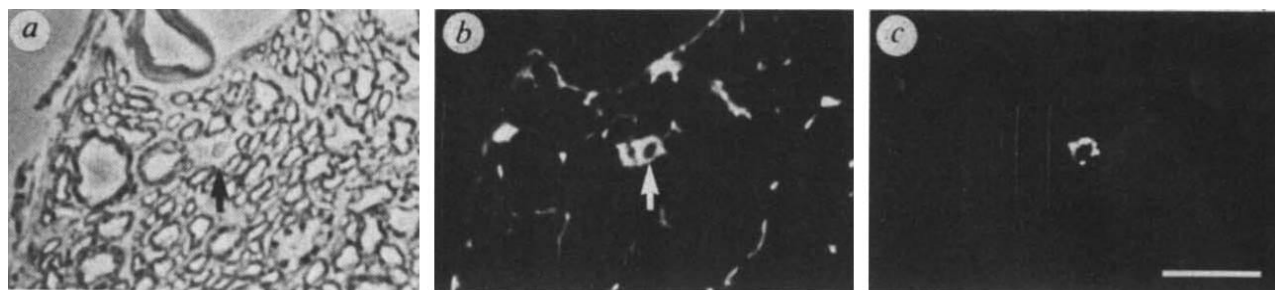


Fig. 1 A transverse semithin frozen section of adult rat optic nerve double-labelled with anti-NSP-4 monoclonal antibody and anti-GFAP serum. *a*, Phase contrast; *b*, rhodamine labelling with anti-GFAP serum; *c*, fluorescein labelling with anti-NSP-4 antibody. The perinodal astrocyte processes surrounding the node of Ranvier (arrow in *a*, *b*) are GFAP⁺ (*b*) and NSP-4⁺ (*c*), while the glial limiting membrane (on the left side of the section) and the large, transversely-orientated astrocyte processes are GFAP⁺ but NSP-4⁻. Note that the small axon immediately to the left of the node is myelinated (*a*) and that the adjacent GFAP⁺, NSP-4⁻ astrocyte process is therefore not perinodal.

Methods. An adult (>3 months) Sprague-Dawley rat was anaesthetised and perfused via the ascending aorta with 4% paraformaldehyde, 0.5% glutaraldehyde and 7% sucrose in 0.1 M phosphate buffer pH 7.4 (PB). The optic nerves were removed and postfixed in the same fixative for 1 h, after which they were immersed in 2 M sucrose in PB for a further hour before being frozen onto copper stubs; 0.5 μ m semithin frozen sections were cut in a freezing microtome and transferred to coverslips as previously described¹⁰. For immunofluorescence double-labelling, sections were initially immersed in normal goat serum diluted 1:2 in PB containing 5% bovine serum albumin, 20 mM lysine and 0.02% (w/v) saponin for 15 min, after which they were sequentially immersed in the specified antibodies, each followed by an appropriate species-specific fluorescent conjugate, diluted in the same buffer, before being mounted, examined in an immunofluorescence microscope and photographed as previously described¹⁰. In the experiment shown here, the sequence of incubations was: anti-NSP-4 antibody¹¹ (culture supernatant diluted 1:2), biotinylated sheep anti-rat immunoglobulin (Sh anti-rat Ig-biotin; Amersham, diluted 1:100), fluorescein-conjugated streptavidin (streptavidin-FI; Amersham, diluted 1:100), rabbit anti-GFAP serum²⁵ (diluted 1:1,000), goat anti-rabbit immunoglobulin conjugated to rhodamine (Nordic, diluted 1:50). In double-labelling experiments to demonstrate the co-distribution of NSP-4 and J1 (not shown), rabbit anti-J1 serum⁹ was used in place of the anti-GFAP serum. All incubations were for 30 min at room temperature except for the anti-GFAP serum for which an overnight incubation at 4°C was used. Control experiments confirmed the species-specificity of the fluorescent conjugates and the absence of labelling when the primary antibodies were omitted. Scale bar, 10 μ m.

double-labelled with anti-NSP-4 antibody and anti-GFAP serum, >99% of the process-bearing, GFAP⁺ type-2 astrocytes¹ were NSP-4⁺, whereas <1% of the flat, non-process-bearing, GFAP⁺ type-1 astrocytes¹ were NSP-4⁺ (Fig. 2*a-c*). The two types of astrocyte in rat optic nerve cultures can be distinguished not only by their morphology but also by the A2B5 monoclonal antibody¹⁴, which recognizes specific gangliosides and labels most type-2 astrocytes but only a small minority of type-1 astrocytes¹. When P1 optic nerve cells were cultured in 10% FCS for 2 or 3 days and then triple-labelled with A2B5, anti-NSP-4 and anti-GFAP antibodies (as described in the Fig. 2 legend), A2B5 labelling was seen on >95% of the NSP-4⁺, GFAP⁺ cells but on <5% of the NSP-4⁻, GFAP⁺ cells, further suggesting that the NSP-4 antigen is found on type-2 but not type-1 astrocytes.

We then cultured optic nerve cells under appropriate conditions to determine if O-2A progenitor cells and oligodendrocytes were NSP-4⁺. First, P1 optic nerve cells were cultured in 10% FCS for 18 hours (by which time very few O-2A progenitor cells have expressed GFAP to become type-2 astrocytes¹⁵), and then double-labelled with anti-NSP-4 and anti-GFAP antibodies; the GFAP⁻ cells with the process-bearing morphology of O-2A progenitor cells¹⁶ were all NSP-4⁺, while the GFAP⁻ flat cells were NSP-4⁻ (Fig. 3). Similarly, when E17 optic nerve cells were cultured for 3 days on type-1 astrocyte monolayers¹⁶ (in order to stimulate proliferation of O-2A progenitor cells¹⁷) clones of bipolar cells characteristic of O-2A progenitor cells¹⁸ were all NSP-4⁺. Finally, P1 optic nerve cells were cultured in 0.5% FCS for 3 days (so that most O-2A progenitor cells differentiated into oligodendrocytes expressing galactocerebroside (GC) on their surface¹⁵), then double-labelled with monoclonal anti-NSP-4 and anti-GC antibodies¹⁹; >90% of the GC⁺ oligodendrocytes were NSP-4⁺ (Fig. 2*d-f*). Thus all cell types in the O-2A lineage express the NSP-4 antigen on their surface in short-term cultures of perinatal rat optic nerve, while the great majority of type-1 astrocytes and non-glial cells do not.

When we labelled P1 optic nerve cells with anti-NSP-4 after 1 day in culture, many of the O-2A progenitor cells were surrounded by a halo of NSP-4⁺ staining (Fig. 3), suggesting that the NSP-4⁺ material was secreted by the cells and was adherent to

the culture substratum. To exclude the possibility that type-1 astrocytes synthesize and secrete NSP-4⁺ glycoproteins but do not bind them to their surface, neonatal optic nerve cultures were grown in 10% FCS for two days, treated with monensin (Sigma, 5×10^{-6} M) for 16 hours (to block the transport of glycoproteins to the cell surface²⁰), and then fixed with acetone before double-labelling with anti-NSP-4 and anti-GFAP antibodies: intracellular vesicular NSP-4 staining was seen in type-2 but not type-1 astrocytes.

These culture experiments suggest that NSP-4⁺ perinodal astrocyte processes in rat optic nerve derive from type-2 rather than type-1 astrocytes. Consistent with this suggestion, type-2 astrocytes appear in the nerve in the second postnatal week⁴, shortly before perinodal astrocyte processes are first seen by electron microscopy²¹. The pattern of NSP-4 antigen expression in developing nerve also supports this suggestion. When semithin frozen sections of optic nerve from P12, P17, P21 and P28 rats were double-labelled with anti-NSP-4 and anti-GFAP antibodies, NSP-4⁺ labelling could be seen within the nerve at all ages, as might be expected from our observation that all cell types of the O-2A lineage are NSP-4⁺ *in vitro*; however few, if any, NSP-4⁺, GFAP⁺ processes were seen in the nerve until P17, when perinodal astrocyte processes (recognized by their characteristic ring-like staining, as shown in Fig. 1) and some fine longitudinally-oriented processes were NSP-4⁺ and GFAP⁺ (Fig. 4). These double-labelled rings increased in number over the next 11 days, as do both type-2 astrocytes⁴ and mature nodes of Ranvier as assessed by electron microscopy²¹.

An alternative explanation for the origin of NSP-4⁺ perinodal astrocyte processes in optic nerve is that type-1 astrocytes, which develop before type-2 astrocytes and largely reach adult numbers by P15⁴, acquire the NSP-4 antigen only after myelination has commenced and then form perinodal processes. Several lines of evidence argue against this interpretation. First, type-1 astrocytes in cultures of P15 optic nerve remained NSP-4⁻ after 2–7 days *in vitro*. Second, in optic nerve cultures, type-2 astrocytes extend fine processes reminiscent of those associated with nodes of Ranvier, whereas type-1 astrocytes do not¹. Third, most type-1 astrocytes go through their final cell division many days before the appearance of perinodal astrocyte processes⁴,

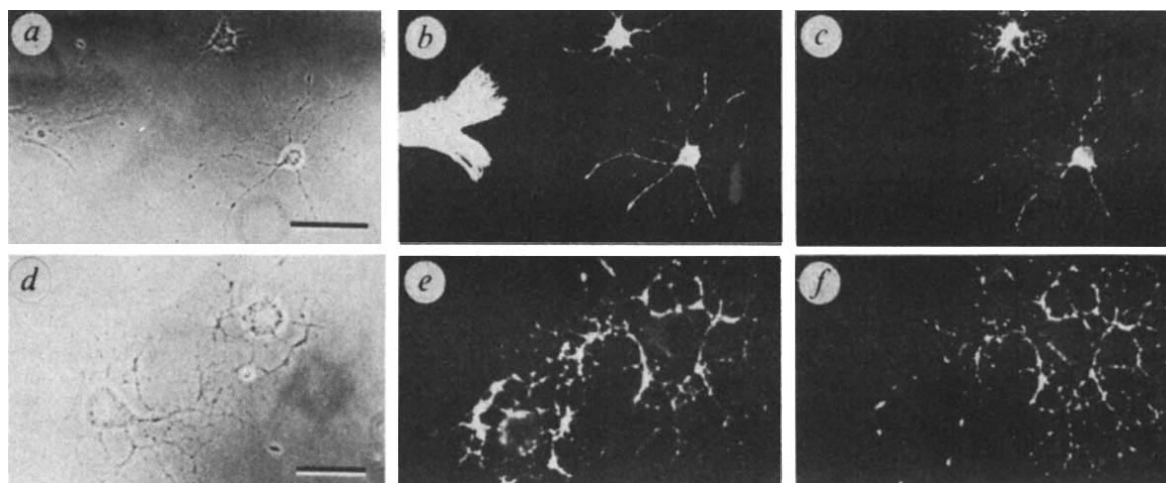


Fig. 2 *a-c*, Three astrocytes in a culture of P7 optic nerve cells grown in 10% FCS for 3 days and then double-labelled with anti-NSP-4 and anti-GFAP antibodies. *a*, Phase contrast; *b*, intracellular fluorescein labelling with anti-GFAP serum; *c*, cell-surface rhodamine labelling with anti-NSP-4 antibody. While the non-process-bearing (type-1) astrocyte on the left and the two process-bearing (type-2) astrocytes on the right are all GFAP⁺ (*b*), only the latter are NSP-4⁺ (*c*). *d-f*, Two oligodendrocytes in a culture of P1 optic nerve cells grown in 0.5% FCS for 3 days and then double-labelled with anti-NSP-4 and anti-GC monoclonal antibodies. *d*, Phase contrast; *e*, fluorescein labelling with anti-GC antibody; *f*, rhodamine labelling with anti-NSP-4 antibody. Both oligodendrocytes (which have a typical multipolar morphology) are GC⁺, and one is also NSP-4⁺. Scale bars: 50 μ m in *a-c*; 25 μ m in *d-f*.

Methods. Optic nerves from perinatal rats were dissociated and cultured on poly-D-lysine-coated coverslips in either Dulbecco's modified Eagle's medium containing 10% FCS or our previously described modification²⁶ of Bottenstein-Sato medium²⁷ containing 0.5% FCS for 1-3 days at 37 °C in a humidified atmosphere containing 5% CO₂ and 95% air as previously described¹⁶. To show the distribution of NSP-4 on astrocytes the cultures in *a-c* were surface-labelled with anti-NSP-4 antibody¹¹ (culture supernatant diluted 1:2) followed by goat anti-mouse immunoglobulin conjugated to rhodamine (G anti-MIg-Rh; Cappel, diluted 1:100), which recognized the rat monoclonal antibody; they were then fixed in acid/alcohol for 10 min at -20 °C, and labelled intracellularly with anti-GFAP serum (diluted 1:1,000) followed by sheep anti-rabbit immunoglobulin conjugated to fluorescein (Sh anti-RIg-FI; Wellcome, diluted 1:100). To show the distribution of NSP-4 on oligodendrocytes two different staining protocols were used, both of which gave identical results; cultures were sequentially surface-labelled either with monoclonal anti-GC antibody¹⁹ (ascites fluid diluted 1:100) followed by immunoglobulin-class-specific goat anti-mouse-IgG3 conjugated to fluorescein (G anti-MIgG3-F1; Nordic, diluted 1:100) and then anti-NSP-4 followed by immunoglobulin-class-specific goat anti-mouse-IgM conjugated to rhodamine (Cappel, diluted 1:25), or alternatively (as in *d-f*) with anti-NSP-4 followed by G anti-MIg-Rh, and then anti-GC followed by G anti-MIgG3-F1; the cultures were then fixed in acid-alcohol. The finding of NSP-4⁺, GC⁻ cells in control experiments in which optic nerve cultures were grown in 10% FCS prior to labelling with the latter protocol confirmed that the G-anti-MIg-Rh did not bind the mouse IgG3 monoclonal anti-GC antibody in the subsequent incubation. For the triple-labelling experiments to demonstrate that NSP-4⁺, GFAP⁺ astrocytes were also A2B5⁺ (not shown) the initial sequence of incubations was A2B5¹⁴ (ascites fluid diluted 1:100), G anti-MIg-Rh, anti-NSP-4, Sh anti-rat Ig-biotin and then streptavidin-FI, after which the cultures were fixed in acid/alcohol before incubation in anti-GFAP serum followed by Sh anti-RIg-FI; the intracellular filamentous GFAP staining could be readily distinguished from NSP-4 surface staining. To prevent the G-anti-MIg-Rh from binding the rat IgM monoclonal anti-NSP-4 antibody in the subsequent incubation, the cultures were incubated in purified mouse immunoglobulin (1 mg ml⁻¹) for 15 min and then fixed briefly in 4% paraformaldehyde in PB before being labelled with anti-NSP-4; in control experiments where an irrelevant rat IgM monoclonal antibody (anti-lyt 2, which recognizes a mouse lymphocyte surface antigen²⁸) was used in place of anti-NSP-4, no cell-surface fluorescein labelling was seen with this protocol. All the antibodies were diluted in minimal Eagle's medium buffered with 0.02 M HEPES and containing 10% FCS, and the cultures were mounted, examined and photographed as previously described²⁶. Control experiments established the specificity of the immunoglobulin-class-specific conjugates, that the Sh anti-RIg-FI did not recognize the anti-NSP-4 or A2B5 monoclonal antibodies, and that the Sh anti-rat Ig-biotin did not recognize the A2B5 mouse antibody.

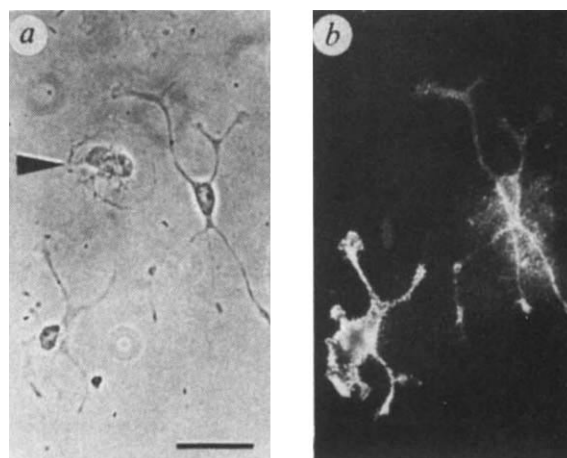


Fig. 3 *a, b*, Two O-2A progenitor cells in a culture of P1 optic nerve cells grown in 10% FCS for 1 day and double-labelled with anti-NSP-4 and anti-GFAP antibodies as described in the legend to Fig. 2. *a*, Phase contrast; *b*, rhodamine labelling with anti-NSP-4 antibody. Both cells with the characteristic process-bearing morphology of O-2A progenitor cells (*a*) are NSP-4⁺ (*b*) and GFAP⁺ (not shown). The O-2A progenitor cell on the right has a halo of NSP-4⁺ secreted material around the cell body. Note that the non-process-bearing cell (arrowhead in *a*) is NSP-4⁻. Scale bar, 25 μ m.

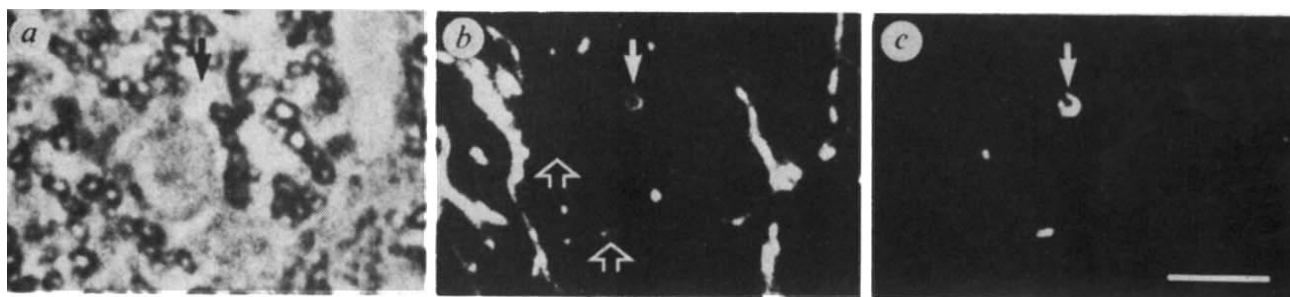


Fig. 4 A transverse semithin frozen section of P17 rat optic nerve showing NSP-4⁺, GFAP⁺ astrocyte processes surrounding a node of Ranvier (arrows). The section was prepared and double-labelled as described in the legend to Fig. 1. *a*, Phase contrast; *b*, rhodamine labelling with anti-GFAP serum; *c*, fluorescein labelling with anti-NSP-4 antibody. Two longitudinal processes are also NSP-4⁺ (*c*) but only one of these is GFAP⁺ (open arrows in *b*). Note that the axonal diameters and extent of myelination are much less at this age than in the adult nerve shown in Fig. 1, where the magnification is half that shown here. Scale bar, 5 μ m.

while in the developing rat spinal cord these processes appear to arise from immature astrocytes, which in some cases have only recently undergone their final division²². Similarly, when we examined P25 optic nerve by electron microscopy, perinodal astrocyte processes were seen that arose from astrocytes with sparse glial filaments, a high density of organelles and other cytoplasmic features of immaturity²³ (C. ff-C, unpublished observations).

As we have previously used the A2B5 monoclonal antibody to label type-2 astrocytes in semithin frozen sections of rat optic nerve⁵, one might expect perinodal astrocyte processes to be A2B5⁺ if they arise from type-2 astrocytes. However, when semithin frozen sections were double-labelled with A2B5 and anti-NSP-4 antibodies, these processes were A2B5⁻. This may be because the A2B5 antigen is concentrated on other parts of the cell; in immunogold electron microscopic studies of prefixed optic nerve cultures, we previously found that the A2B5 antibody labelled type-2 astrocytes mainly on their cell bodies while processes remained largely unlabelled³.

In the present study we have shown that in cultures of perinatal rat optic nerve, labelling by anti-NSP-4 antibody is largely confined to the O-2A cell lineage, being found on O-2A progenitor cells, oligodendrocytes and type-2 (but not type-1) astrocytes. While the same was true for anti-J1 serum (not shown), this antiserum often labelled secreted material⁹ to a greater extent than anti-NSP-4, making interpretation difficult; we therefore used the latter antibody for these experiments. Since all GFAP⁺ perinodal astrocyte processes are apparently NSP-4⁺ (and J1⁺)¹⁰, our present results suggest that these processes are derived primarily, and perhaps exclusively, from type-2 astrocytes. In light of this, it seems reasonable to view the O-2A lineage as specialized for constructing myelin sheaths and nodes of Ranvier in the CNS. This view provides a plausible explanation for why type-2 astrocytes are more closely related in origin to oligodendrocytes than to type-1 astrocytes. A relationship between cell lineage and function is also seen in the haemopoietic system, where the two types of 'professional' phagocytes, neutrophils and macrophages, develop from a common progenitor cell²⁴.

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Identity of differentiation inducing factor and tumour necrosis factor

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Human myelogenous leukaemic cells can be induced to differentiate into the monocyte/macrophage pathway by protein inducers called differentiation inducing factors (DIF) in conditioned media of mitogen-stimulated human peripheral blood leukocytes¹⁻⁸. However, human DIF has not yet been well characterized. DIF is known to be a T-cell lymphokine, as it can be obtained from the T-cell line HUT-102⁷ and can be partially purified from medium conditioned by phytohaemagglutinin (PHA)-stimulated lymphocytes⁸. We found that monocytes also produce factor(s) that induce differentiation of human myelogenous leukaemia cell lines to cells with macrophage-like characteristics. This factor(s) has activity different from that of colony-stimulating factor(s) or interferons⁹. We have now purified a DIF to homogeneity from medium conditioned by PHA-stimulated leukocytes using a human myeloblastic leukemia cell line, ML-1¹⁰, as target cells. The purified DIF

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has a relative molecular mass (M_r) of approximately 17,000, with an NH_2 -terminal sequence the same as that of human tumour necrosis factor (TNF). Recombinant human TNF (rHuTNF) induces differentiation of ML-1 cells and an anti-pDIF monoclonal antibody can neutralize both differentiation inducing activity and cytotoxic activity of DIF and rHuTNF. The findings indicate that one of the DIF(s) produced by leukocytes is probably TNF.

A differentiation inducing factor (DIF) was purified to homogeneity from medium conditioned by human peripheral blood mononuclear cells stimulated by PHA. Activity of this DIF was assayed by measuring reducing activity of ML-1 cells by nitro blue tetrazolium (NBT) dye, since this reducing activity was associated with induction of other phenotypic markers of cell differentiation such as phagocytic activity and morphological changes.

Human peripheral blood mononuclear cells were resuspended at $1-2 \times 10^6$ cells ml^{-1} in RPMI 1640 medium containing 5% heat-inactivated human serum and PHA-P (Difco) $10 \mu\text{g ml}^{-1}$ and incubated at 37°C for 3 days. After removal of cells and debris, the supernatant was concentrated by the Pellicon cassette system (Millipore) and passed through an affinity column of anti-interferon- γ (IFN- γ) monoclonal antibody to remove IFN- γ . The preparation was then purified further in a process including batched silica gel chromatography, DEAE-Sepharose chromatography, phenylalanine-Sepharose chromatography, Ultragel Aca 44 chromatography and chromatofocusing.

The purified protein was homogeneous according to the criteria of SDS-polyacrylamide gel electrophoresis, had a M_r of 17,000 and was associated with all differentiation-inducing activity (Fig. 1). Homogeneity was also confirmed by reverse-phase high performance liquid chromatography (HPLC). Purified DIF activity was eluted in 67% acetonitrile in reverse-phase HPLC as a single sharp peak coincident with DIF activity (Fig. 2).

We obtained specific activity of 1×10^5 U per mg of protein (1 U DIF induced 50% of maximum induction as indicated by reduction of NBT) and final purification of 4,700-fold, calculated from the first supernatant concentration. The overall yield was 0.5%. The purified DIF induced maturation of the ML-1 cells along the monocytic cell pathway. The morphology of untreated ML-1 cells was characterized by a relatively large nucleus with scant cytoplasm (Fig. 3a), while the ML-1 cells treated with the DIF were changed to macrophage-like cells that had granules in abundant cytoplasm, and an eccentrically placed oval nucleus with loosely stranded nuclear chromatin

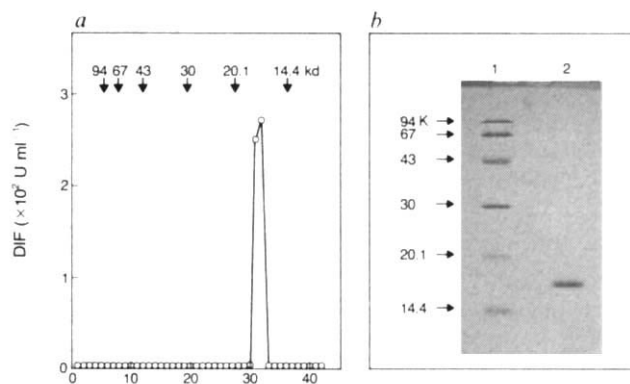


Fig. 1 Profile of DIF activity (a) and Coomassie brilliant blue stained gel pattern after SDS-polyacrylamide gel electrophoresis (b). Polyacrylamide-SDS gels (15%) were run as described by Laemmli²². Purified DIF sample was dissolved in 2% SDS sample buffer containing no mercaptoethanol and stood at room temperature for 30 min. The gels were electrophoresed at 10–30 mA per gel. M_r markers were phosphorylase, 94,000; bovine serum albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 20,100; and α -lactalbumin, 14,400. Gels were cut to remove sample tracks for NBT assay while the rest of the gel was fixed and stained with Coomassie brilliant blue. Each sample track was cut into 2-mm slices and each slice was extracted overnight into 1 ml of RPMI 1640 medium containing 10% fetal bovine serum at 4°C . Of each fraction, 50 μl was added to ML-1 cells derived from a patient with human myeloblastic leukaemia, in a 96-well microplate, to a final volume of 100 μl (3×10^4 cells) per well. After 3 days, the capacity to reduce nitro blue tetrazolium (NBT) was determined. After adding 50 μl of NBT solution (RPMI 1640 medium containing 10% FBS, 0.2% NBT, and 40 ng TPA), the preparation was incubated at 37°C for 30 min. Cells with blue-black formazan deposits were dissolved in DMSO, and colour absorbance was measured at A_{590} by a Titertec Multiscan Autoreader (Flow). Units of activity was calculated from assays on serial 2-fold dilution of test material. One unit was defined as 50% of maximum inducing activity by ML-1 cells as indicated by reduction of NBT.

Table 1 Antigenic similarity of DIF and rHuTNF as assessed by neutralization of reduction of NBT

Addition	Anti-DIF monoclonal antibody (2H-4)	% NBT reducing cells
None	–	2
	+	3
DIF, 2 U ml^{-1}	–	60
	+	18
rHuTNF, $5 \times 10^{-11} \text{ M}$	–	57
	+	14

Neutralizing monoclonal antibody was derived from mice immunized with DIF. $10 \mu\text{g}$ DIF from a chromatofocusing fraction was primed by intraperitoneal and subcutaneous injection in aluminium hydroxide gel. Three weeks later they were inoculated similarly but with incomplete adjuvant. Spleens were removed 3 days after final inoculation and fused with the myeloma line (X63-Ag8-6.5.3) following a previously described procedure²⁴. The fused cells were distributed in 192 wells (96-well plates) in Dulbecco's modified Eagle's medium supplemented with 10% horse serum and hypoxanthine/aminopterin/thymidine (HAT) components. Hybrid cultures that were positive in the neutralization assay against differentiation inducing activity were used. DIF or TNF samples were preincubated with anti-DIF monoclonal antibody (2H-4) at 37°C for 60 min before assay on ML-1 cells. After 3 days, the capacity to reduce NBT was determined as described in Fig. 1.

(Fig. 3b). The lineage-specific differentiation induced by the DIF was confirmed by lineage-specific esterase stain. Untreated ML-1 cells reacted only weakly to the α -naphthyl acetate esterase stain, whereas the number of cells that responded strongly to this stain increased after DIF treatment. No colony-stimulating activity or anti-virus activity was detected in the purified fraction, but cytotoxic activity was detected. Fifty per cent of L-929 cells were lysed in the presence of 0.05 U ml^{-1} of DIF and $1 \mu\text{g}$ actinomycin D. The homogeneity of the material after final purification suggests that these two biological activities reside in the same molecule.

N-terminal sequence analysis of DIF showed a striking homology with the N-terminal sequence of human TNF¹⁴. The first 20 residues of each were the same, $\text{H}_2\text{N-Val-Arg-Ser-Ser-Ser}^5\text{-Arg-Thr-Pro-Ser-Asp}^{10}\text{-Lys-Pro-Val-Ala-His}^{15}\text{-Val-Val-Ala-Asn-Pro}^{20}$. TNF was so named because this molecule induced haemorrhagic necrosis in certain animal tumours and in some heterotransplanted human tumours^{15,16}. It also exhibited a cytostatic and cytolytic activity against transformed cell lines in culture^{15–18}. These results further suggest that DIF and TNF are the same molecule. For this reason we examined the differentiation inducing activity of recombinant human TNF (rHuTNF). Differentiation of ML-1 cells into cells with macrophage characteristics the same as those of DIF was induced by rHuTNF. The molar concentration of TNF required to elicit differentiation was less than $1 \times 10^{-12} \text{ M}$. At a TNF concentration of $5 \times 10^{-11} \text{ M}$, NBT reduction was induced in 57% of ML-1 cells.

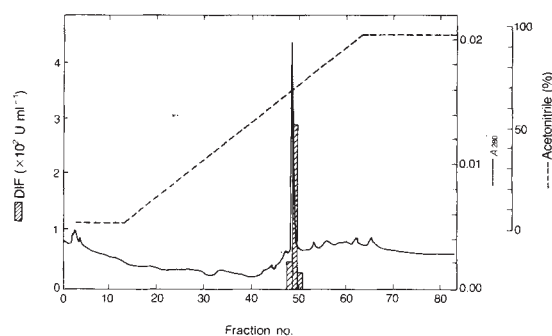


Fig. 2 Reverse-phase HPLC of DIF. In a high performance liquid chromatography (HPLC) system (Waters), 20 μ g DIF from electrofocusing fractionation was applied to the reverse-phase column, TMS-250 (Toyosoda). The column was equilibrated with water containing 0.05% trifluoroacetic acid and eluted through a 60-min linear time gradient with 95% acetonitrile (v/v) containing 0.05% trifluoroacetic acid. The flow rate was maintained at 0.6 ml min⁻¹ and 0.6-ml fractions were collected. NBT reducing activity was assayed by the method described in Fig. 1.

To confirm the identity of both of these molecules we prepared anti-DIF mouse monoclonal antibody (MoAb2H4), which neutralized the NBT reducing activities of both DIF and rHuTNF (Table 1). It also neutralized the cytotoxic activity of both molecules against mouse L-929 cells. One of the DIF(s) produced by leukocytes in the presence of PHA-P could thus be identified as TNF. DIF(TNF) induced differentiation of ML-1 cells (Fig. 3a, b); of HL-60, a human promyelocytic leukaemia cell line; and of THP-1, a human monoblastic leukaemia cell line (Fig. 3c, d). However, DIF had no effect on the differentiation of K-562, a human erythroblastic leukaemia cell line.

Recently Beutler *et al.* showed that 'cachectin' had a high degree of sequence homology to human TNF¹⁹. When macrophages were stimulated with an endotoxin, they produced a factor, cachectin, which inhibited the activity of fat-producing (lipogenic) enzymes and their gene expression in cultured adipocytes^{20,21}.

The results presented here point to another new biological effect of TNF. We believe there could be a system of feedback control of monocytic cell maturation. Activated macrophages

produce TNF(DIF) which not only cytolyse the transformed cells or acts like cachectin in changing activity of lipogenic enzymes, but also enhances differentiation of the progenitor cells of the macrophages. This could be one of the regulatory signals that control cellular reactions during infection. It remains to be clarified how TNF(DIF) can induce differentiation of certain cell lines while killing others.

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Phenotypic changes induced by a mutated *ras* gene during the development of *Dictyostelium* transformants

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The *ras* proto-oncogene¹, found in all eukaryotes so far examined²⁻⁹, encodes a protein with guanine nucleotide-binding and GTPase activity¹⁰⁻¹³. Gene disruption experiments in yeast indicate that *ras* is essential for cell growth¹⁴. Anti-sense mutagenesis approaches suggest that this is also true for *Dictyostelium*¹⁵. Most mutations causing an amino-acid substitution for Gly 12 result in decreased GTPase activity and produce a transforming phenotype¹⁶⁻¹⁹. In yeast, a Gly 19 → Val 19 missense mutation (Gly 19 is similar to Gly 12 in mammalian and *Dictyostelium* *ras* proteins) causes a series of dominant phenotypes, including elevated adenylate cyclase activity²⁰. In mammalian cells there is no evidence that *ras* activates adenylate cyclase activity²¹. *D. discoideum* contains a single *ras* gene (*Dd-ras*) that encodes a protein very similar to the mammalian *ras* protein⁴ and identical

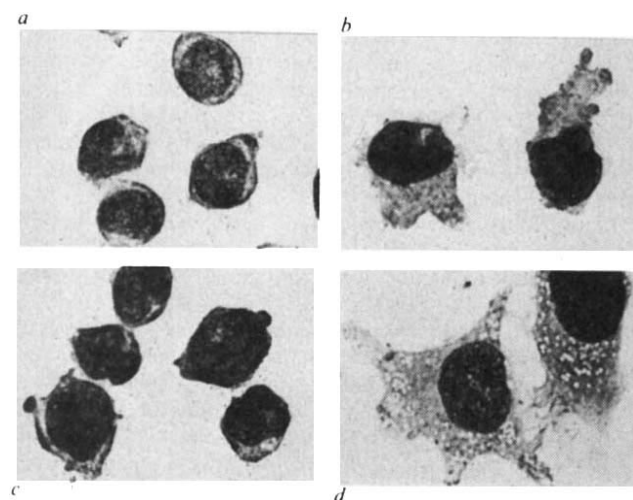


Fig. 3 Morphological changes. ML-1 cells or THP-1 cells²³ were cultured for 5 days in the presence of 5×10^{-11} M TNF in tissue culture chamber/slides (Lab-Tek) and stained with May-Grunwald-Giemsa ($\times 1,000$). a, Uninduced ML-1 cells. b, ML-1 cells treated with TNF. c, Uninduced THP-1 cells. d, THP-1 cells treated with TNF.

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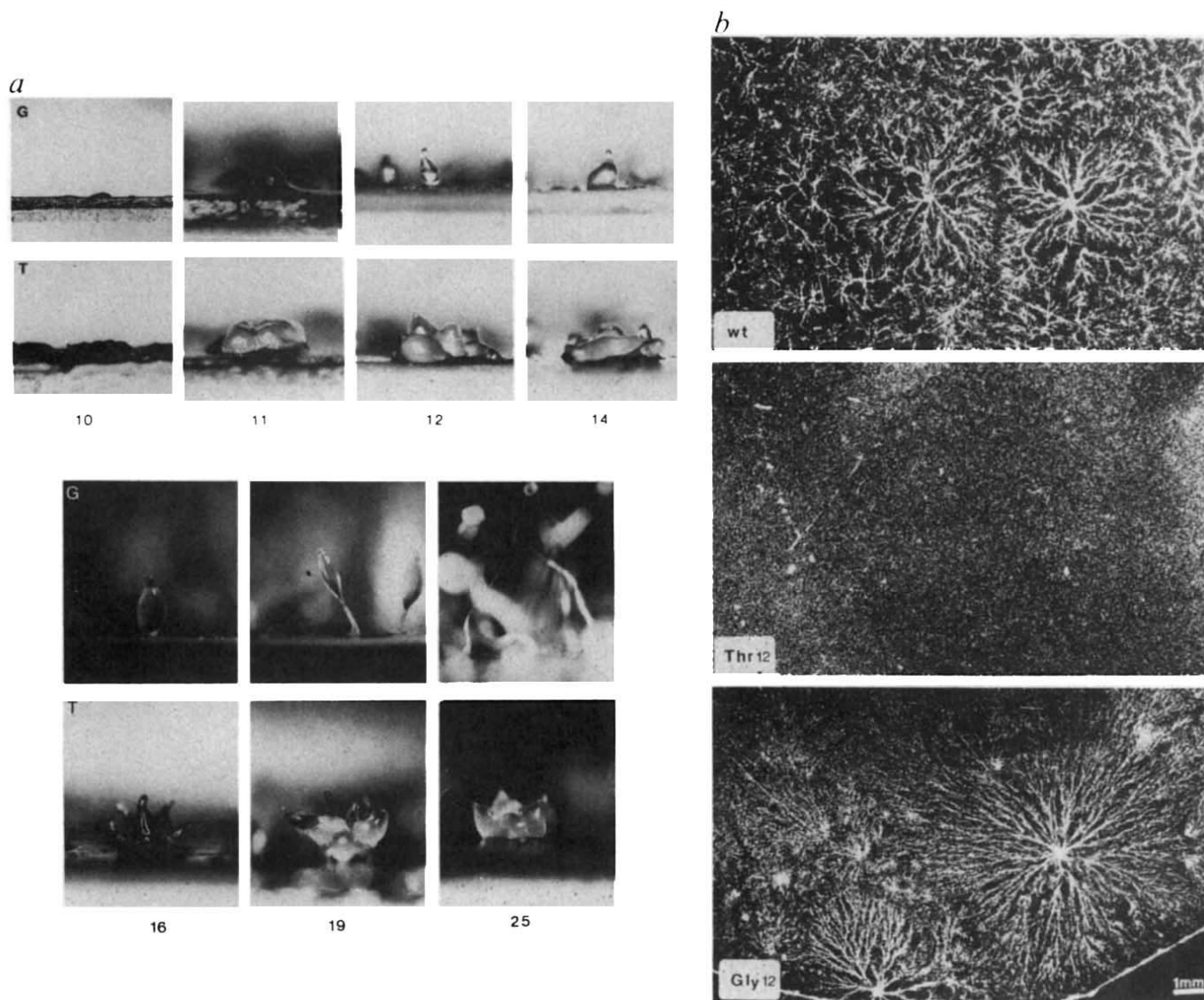


Fig. 1 Comparative phenotype of *ras* transformants. *a*, Aggregations of *ras*-Gly 12(G) and *ras*-Thr 12(T) transformants, photographed at indicated times (h). 11, tight aggregation; 14, slug formation; 16–25, culmination for KAX-3 cells. *b*, Organized streams of cells. wt, Wild type.

Methods. The *ras*-Gly 12 and *ras*-Thr 12 genes in the B10A transformation vector^{15,23} were constructed as follows. The complementary DNA *Dd-ras* Cl (ref. 4) was cloned into M13mp9, which provided a *Hind*III site 3' to the cDNA within the polylinker. A *Dde*I site within the cDNA was used to fuse the cDNA in-frame to the genomic *Dd-ras* gene. Previous work¹⁵ suggests that the region between the *Cl*aI site and translational start carries all the necessary *cis*-acting sequences for proper developmental regulation of the *ras* gene. A 20-mer oligonucleotide GTAGGTGGTACTGGTGTGG (altered nucleotides underlined) was used for the *in vitro* site-directed mutagenesis³⁵ on single-stranded phage DNA from a M13mp11 clone containing a *Cl*aI-*Pst*I fragment obtained from a previous construct (construct A₃ in ref. 15). The M13 plaques obtained were screened with 5' end-labelled 20-mer oligonucleotide³⁶ and single-stranded DNA from positive phage was sequenced³⁷. The appropriate fragment from a *Cl*aI partial *Hin*FI digest was used to replace the mutated fragment in the vector to make a *ras* gene (either Gly 12 wild-type or Thr 12) carrying the entire coding region and all the essential 5' flanking sequences (ref. 15; P. Howard, C.D.R. and R.A.F., unpublished data) for proper regulation in vector B10A. This *ras* gene construct does not contain a transcription termination/polyadenylation site; however, previous work has shown that when it is placed 5' to the *Act6-neo*^R gene fusion the 5' flanking region of the actin 6 gene provides such a sequence^{15,24}. These *ras* constructs in the B10A transformation vector produce RNAs now distinguishable in size from the endogenous *ras* mRNA. *a*, *Ras*-Gly 12 and *ras*-Thr 12 cells were grown in HL5 medium containing G418 at 5 µg ml⁻¹, washed and allowed to develop synchronously on nitrocellulose filters (10⁸ cells per 5.5-cm diameter filter)²² and individual aggregates photographed at the indicated times. The *ras*-Gly 12 transformants were indistinguishable from wild-type KAX-3 aggregates (not shown). *b*, Transformants (*ras*-Gly 12 and *ras*-Thr 12) and KAX-3 cells were washed and 2 × 10⁶ cells were plated in MES-PDF buffer in a 3.5-cm tissue-culture plate³⁸. Pictures taken after 8 h of incubation. Both KAX-3 (wt) and *ras*-Gly 12 cells formed organized streams of cells migrating toward aggregation centres. Only small aggregates can be observed with *ras*-Thr 12-transformants.

to *c-ras* at the potentially transforming positions. *Dd-ras* is expressed in vegetative cells and later in development in prestalk cells whereas *ras* protein is found in vegetative and developing cells. In the migrating pseudoplasmodium, *ras* protein is found in prestalk but not prespore cells, suggesting it is involved in the function and/or differentiation of the anteriorly localized prestalk cells. In this report we examine the effects of expression of a *Dd-ras* gene carrying a Gly 12 → Thr 12 missense mutation.

A Gly 12 → Thr 12 missense mutation in *Dd-ras* was made by oligonucleotide-directed mutagenesis. *Dictyostelium* cells were

transformed in parallel with the transformation vector B10A lacking a *ras* gene, B10A-*ras*-Gly 12, or B10A-*ras*-Thr 12. All transformants grew at the same rate in medium containing G418 although the transformation frequency was appreciably lower (>10-fold) with B10A-*ras*-Thr 12. When *ras*-Thr 12 transformants were plated on SM agar plates in association with *Klebsiella aerogenes*²², 70–90% of the colonies formed aggregates with multiple tips whereas *ras*-Gly 12 and B10A transformants formed normal aggregates with single tips indistinguishable from the parental strain KAX-3. When developed

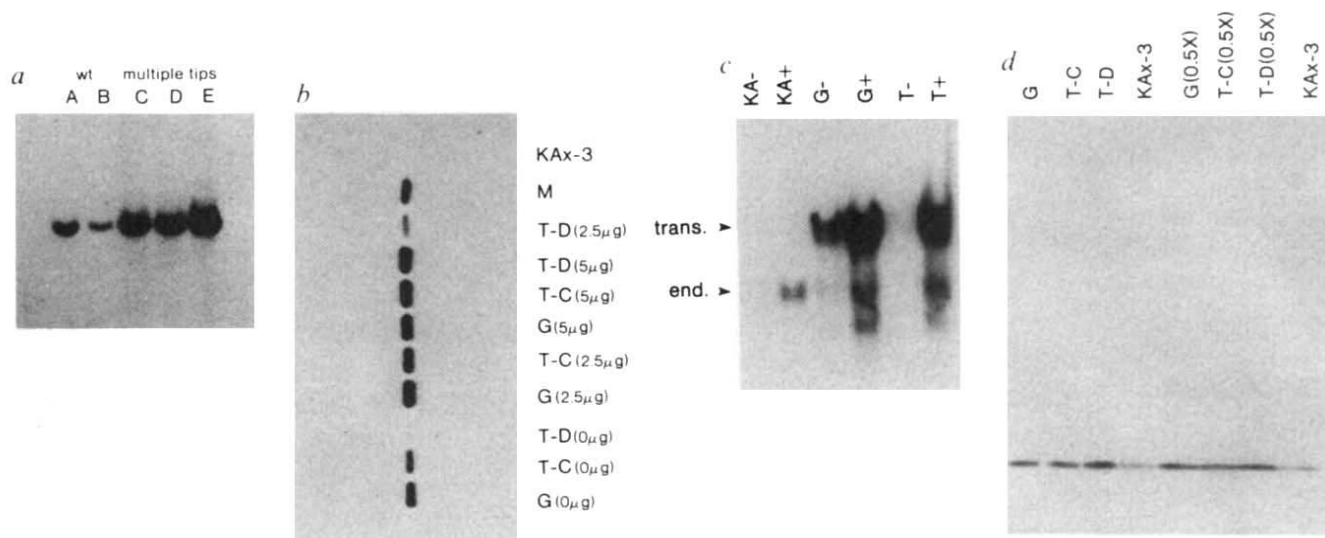


Fig. 2 *a*, *b*, DNA; *c*, RNA and *d*, protein analysis of *ras* transformants. *a*, Single colonies from the *ras*-Thr 12 transformants were picked and DNA was isolated from cells. Approximately 3 μ g of each DNA was digested with *Cla*I and *Hind*III (that excises the transformed *ras* gene), separated on an agarose gel and blotted onto nitrocellulose membrane³⁶. The filter was hybridized with nick-translated *Dd-ras*Cl cDNA^{15,32}. A more intense hybridization of the 1.6-kilobase (kb) DNA fragment from the transfected *ras* genes is observed with DNA from colonies showing multiple tips (lanes C, D, E) than with colonies which developed normally (lanes A, B). wt, Wild-type (single tipped aggregates). *b*, DNA from *ras*-Gly 12 (G) and the *ras*-Thr 12 (T) transformants corresponding to lanes C and D in *a* (respectively, lanes T-C and T-D) was analysed as described in *a*. Transformed cells growing under G418 selection were separated in three aliquots, one kept under selection at 2.5 μ g or 5 μ g ml⁻¹ G418 (lanes 2.5 and 5 μ g) and one grown without G418 (lanes 0 μ g). DNA was isolated after 5 days in culture with 2.5 μ g ml⁻¹ G418 selection or 14 days with 5 μ g ml⁻¹ G418 or without G418. A lower copy number of the vector is observed in cells grown without selection (compare lanes 0 with 5 μ g). The genomic 2.9-kb *ras* gene fragment can be seen upon longer exposure in KAx-3 cells (data not shown). Lane M, DNA from *ras*-Gly 12 vector loaded at an equivalent of 100 copies per cell. *c*, Induction of prestalk mRNAs by cAMP in fast-shaking culture was examined as previously described^{4,15,39}. Cells from untransformed KAx-3 (KA), *ras*-Gly 12 transformants (G), and *ras*-Thr 12-transformants (T) were washed and starved in fast-shaking culture for 6 h and cAMP added to 300 μ M to + cultures and no cAMP to - cultures. Levels of cAMP were maintained in + cultures. RNA was extracted after 12 h, sized on denaturing gels, blotted and probed with *ras* cDNA^{4,15}. end, Endogenous 1.2-kb *ras* mRNA; trans, the mRNA from the *ras*-Thr 12 and *ras*-Gly 12 genes. The basal level of prestalk mRNAs in - cAMP cultures is variable and a function of the extent of cell-cell contact. In these experiments G- is high. Another prestalk gene (16-GI) (see ref. 39) was also assayed and shows parallel levels of expression to *ras* (data not shown). *d*, Proteins from transformants grown in 5 μ g ml⁻¹ G418 and KAx-3 cells as described in *b* were separated on polyacrylamide gels, blotted to nitrocellulose and stained with a polyclonal anti-*Dd-ras* antibody⁴. Twofold less protein was loaded in lanes 0.5X. Comparison of lanes containing protein from transformants with lane KAx-3 indicates that transformants grown under G418 selection produce 2-4 $\times$ more *ras* protein than untransformed KAx-3 cells.

on Millipore filters, *ras*-Thr 12 transformants form multiple-tipped aggregates that do not fruit while *ras*-Gly 12 transformants proceed through development in a manner indistinguishable from wild-type KAx-3 cells (Fig. 1*a* and data not shown). In a submerged culture assay (ref. 25; Fig. 1*b*), *ras*-Thr 12 transformants do not form aggregation streams whereas control KAx-3 cells and *ras*-Gly 12 transformants form large aggregation streams at similar cell densities. At a 2.5-fold higher cell density, very small, star-like aggregates form (<1/20 diameter of streams shown in Fig. 1*b*), but only after a prolonged incubation time (data not shown).

Cells from single colonies grown in 5 μ g ml⁻¹ G418 showing the multiple-tip phenotype have a fivefold higher copy number of the vector than colonies not exhibiting the phenotype (Fig. 2*a*). Vector copy number decreases when cells are grown in 2.5 μ g ml⁻¹ G418 or without G418 selection (see Fig. 2*b*). This instability in vector DNA contrasts with that observed for all other integrated vectors yet examined^{23,24}, suggesting there is strong selection against the *ras*-Thr 12 gene. Colonies having lost or reduced levels of this gene show wild-type phenotypes, indicating a dependence of the phenotype on *ras*-Thr 12 copy number.

In vegetative transformed cells, the steady-state levels of the messenger RNA from the ~100 transformed genes are ~20-fold higher than that of the endogenous gene (data not shown). The difference between relative copy number and mRNA levels could be caused by a lower mRNA stability because of a different 3'

end, or possibly a lower promoter activity of the transfected gene. Expression of *ras*-Gly 12 and *ras*-Thr 12 genes parallels that of the endogenous *ras* gene during development (data not shown) and the expression of two different endogenous prestalk and prespore genes is unaltered in transformants. The endogenous *ras* gene and both transfected *ras* genes, like other prestalk-specific genes, are induced in developing single cells in suspension culture by the addition of cyclic AMP (see Fig. 2*c*), as previously shown with other constructs using the *Dd-ras* promoter^{4,15}.

Total *ras* protein levels are about fourfold higher in the high-copy *ras*-Thr 12 and *ras*-Gly 12 transformants than in control cells (Fig. 2*d*). It is not possible to distinguish between the endogenous *ras* protein and that encoded by the transfected gene. Because of the high level of transfected *ras* gene mRNA, we expect that a major portion of *ras* protein is encoded by the transfected gene. Transformed cells, with fewer vector DNA copies and a wild-type phenotype, contain *ras* protein levels indistinguishable from non-transformed cells (data not shown). It is not known why the relative elevation of *ras* protein levels is less than that of the mRNA levels.

Aggregation in *Dictyostelium* is brought about by chemotaxis and cAMP signalling via a cell-surface cAMP receptor-mediated, GTP-dependent activation of adenylate cyclase²⁵. It has been suggested that cAMP is important in cellular morphogenesis during multicellular aggregation and in cell-type gene expression²⁶⁻²⁹: multitipped aggregates can be produced by the

Table 1 Adenylate cyclase activity in *Dictyostelium* transformants

Experiment/strain	Basal*	Adenylate cyclase activity (pmol cAMP min ⁻¹ mg protein ⁻¹)		Mn ²⁺
		GppNp alone <i>in vitro</i>	cAMP+ GppNp <i>in vitro</i>	
Expt 1				
<i>ras</i> -Thr 12	9.3	ND	68.2	53.2
<i>ras</i> -Gly 12	10.2	ND	74.7	94.7
Expt 2				
<i>ras</i> -Thr 12	5.4	ND	51.5	15.8
<i>ras</i> -Gly 12	2.2	ND	43.4	15.2
Expt 3				
KAx-3	3.8	15.0	41.7	19.2
<i>ras</i> -Thr 12	9.4	15.0	49.0	16.7

Adenylate cyclase activity was measured in 6-h developing cells as described in ref. 34. *Dictyostelium* cells were filter lysed in the conditions indicated, preincubated for 5 min at 0 °C and assayed for 1 min at 22 °C. GppNP 50 µM, cAMP 1 µM and MnSO₄ 5 mM. ND, not determined. KAx-3 cells are parental cells for the transformants. The *ras*-Thr 12 transformant used here (isolate C) is the same transformant used in experiments described in previous figures.

* Variation in basal adenylate cyclase observed in these experiments is within the range observed for wild-type cells in 17 separate experiments and is not considered significant (see ref. 34).

addition of cAMP to developing aggregates³⁰. Because the phenotypes of the *ras*-Thr 12 mutants suggest that cAMP signalling is impaired in these cells, we examined cAMP receptors and adenylate cyclase levels in *ras*-Thr 12-transformants and wild-type cells.

Scatchard plot analysis (data not shown) shows that receptor levels at 6 h of development are indistinguishable in KAx-3 cells and *ras*-Thr 12 and *ras*-Gly 12 transformants. In *Dictyostelium*, the affinity of cAMP for its surface receptor in isolated membranes is reduced by GTP, GppNp and GDP³¹, probably via interaction of the receptor with G-proteins. We measured ligand affinity and found no difference in cAMP binding to the receptor in the presence of guanine nucleotides in membranes from *ras*-Thr 12 and KAx-3 cells (data not shown), suggesting either that *ras* protein does not mediate this affinity directly or that the mutation does not alter this function. In addition, cAMP-induced receptor phosphorylation is unaffected in the transformants (data not shown).

Adenylate cyclase levels were also measured in the presence of Mn²⁺ (uncoupled) or Mg²⁺ plus cAMP and GppNp. Adenylate cyclase is activated in aggregation-competent cells *in vivo* by cAMP binding to the receptor that is activated *in vitro* by guanylnucleotide triphosphates³³ and enhanced by cAMP two- to fourfold. Addition of cAMP plus GppNp leads to an average 10-fold stimulation over basal cyclase activity in the wild-type strain KAx-3 at 0 h (not shown) or 6 h in development and that there is no observable difference between *ras*-Thr 12 and wild type (Table 1). Unregulated activity (in the presence of Mn²⁺) is unaltered in the *ras* transformants. The slight differences in absolute activities probably result from developmental differences, variations in receptor number and endogenous GTP and cAMP stimulation of basal activity. We also examined adaptation of the adenylate cyclase following *in vivo* cAMP treatments in the transformants and found that neither the rate nor the extent of adaptation is different.

Expression of a Gly 12 → Thr 12 *Dd-ras* missense mutation in *Dictyostelium* transformants results in cells with aberrant developmental phenotypes suggestive of an alteration in the cAMP mediated signal transduction pathway. Over-expression of *Dd-ras*-Gly 12 has no observable phenotype. The cAMP receptor levels, effect of guanine nucleotides on the affinity of cAMP for the receptor, ligand-induced receptor phosphorylation, and cAMP- and guanylnucleotide-dependent activation and desensitization of adenylate cyclase are not substantially altered in these transformants. But small aberrations in any of these biochemical events that may not be detectable *in vitro* could be sufficient *in vivo* to result in the observed phenotypes. For instance, chronic weak inhibition (~10%) of the signalling response will alter aggregation morphology, and cAMP binding to the receptor also results in a very rapid transient increase in Ca²⁺ influx^{25,32} and cGMP levels (see ref. 25), as well as phosphorylation of myosin heavy and light chains³³. It is possible that *ras* directly or indirectly regulates one of these events, and also plays a part in modulating cellular events other than those regulated via the cAMP receptor. Analysis of *Dd-ras* should help to elucidate mechanisms regulating cellular differentiation in *Dictyostelium* as well as the functions of *ras* in higher eukaryotes.

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T-cell responses to human AIDS virus in macaques immunized with recombinant vaccinia viruses

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There is much interest in developing vaccines against acquired immune deficiency syndrome (AIDS), which is caused by a retrovirus termed human immunodeficiency virus (HIV)¹. Isolates of this virus include human T-lymphotropic virus type III (HTLV-III)², lymphadenopathy-associated virus (LAV)³, and AIDS-associated retrovirus (ARV)⁴. Several approaches towards the development of an AIDS vaccine result in the production of antibodies in subprimates. These methods involve the use of: (1) antigens isolated from the AIDS virus; (2) viral antigens expressed by transfected cells⁵ or by recombinant vaccinia viruses^{6,7}; and (3) particular synthetic peptides of viral antigens⁸. Because T-cell-mediated immunity (in addition to antibodies) is involved in resistance to diseases and death caused by various enveloped viruses⁹⁻¹⁶, we sought to determine whether potential AIDS vaccines can induce T-cell responses against the AIDS virus. Here we report that immunization of non-human primates, *Macaca fascicularis* (macaques), with recombinant vaccinia viruses that express LAV envelope glycoproteins gp41 and gp110 results not only in the production of antibodies against the LAV envelope antigens but also in the generation of T-cells that proliferate and produce the lymphokine interleukin-2 (IL-2), in response to stimulation with purified LAV. We believe this is the first report demonstrating T-cell-mediated immunity to the virus that causes AIDS.

We recently reported the construction of a recombinant vaccinia virus that expresses the envelope glycoproteins of LAV and induces antibodies to these glycoproteins in immunized mice⁶. To show that this recombinant, v-env5, also induces LAV-specific immune responses in primates, we immunized eight macaques intradermally by local scarification on the mid-line of the back with 2×10^7 or 2×10^8 plaque-forming units (PFU) of the recombinant virus and then boosted 7 of them 12 weeks later. Four weeks after the boost, serum samples were collected and analysed for reactivity with LAV envelope glycoproteins by Western blots. Sera from all seven of these boosted macaques show strong reactivity with gp41 (Fig. 1b) and sera from four of these also show strong reactivity with gp 110 (Fig. 1a). Serum from macaque 81, which received a single inoculation with v-env5, shows weak reactivity to both glycoproteins. In contrast, no reactivity with gp41, gp110 or the precursor glycoprotein gp150 is detected with sera from pre-immune or other non-immunized macaques or with serum from macaque 73, which was immunized twice with a control recombinant vaccinia virus (v-HSVgDI) that we previously constructed and showed to express the glycoprotein gD of herpes simplex virus type 1 (HSVgDI) (S.-L. Hu, unpublished data). Serum from the v-HSVgDI-immunized macaque does, however, react with HSV-1 and this serum, as well as sera from the v-env5 immunized macaques, also reacts with parental vaccinia virus (data not shown).

To determine whether T-cell-mediated immune responses to AIDS virus can also be detected in these vaccinated macaques, we tested peripheral blood lymphocytes (PBL) for their ability to proliferate and incorporate ³H-thymidine in response to stimulation with purified, non-disrupted LAV. PBL from all 8

Table 1 LAV-induced proliferative responses of PBL from macaques after immunization with a vaccinia recombinant virus expressing LAV envelope glycoproteins

Macaque No.		Virus used for PBL stimulation		
		Immunization	None	LAV
		c.p.m. ³ H-TdR incorporated (stimulation index)		
67	v-env5	1,586	7,465(4.7)	60,415(38.1)
68	v-env5	2,245	9,075(4.0)	28,638(12.8)
74	v-env5	1,585	4,732(3.0)	21,487(13.6)
75	v-env5	581	8,645(14.9)	37,847(14.1)
76	v-env5	2,479	5,987(2.5)	36,657(14.8)
80	v-env5	1,077	13,922(12.9)	24,752(23.0)
81	v-env5	965	3,985(4.1)	40,572(42.0)
82	v-env5	2,581	8,580(3.3)	46,847(18.1)
73	v-HSV-gDI	612	553(1.0)	56,217(91.9)
26	none	2,911	1,811(<1.0)	2,240(<1.0)
27	none	1,228	1,072(<1.0)	2,532(2.0)

PBL were isolated by Ficoll-hypaque centrifugation from heparinized blood of macaques 4 weeks after a second intradermal immunization with a vaccinia recombinant virus expressing LAV glycoproteins gp41 and gp110 (v-env5), a vaccinia recombinant virus expressing HSVgDI and from non-immunized macaques. PBL were also isolated from macaque 81 which was vaccinated only once with v-env5. Both recombinant vaccinia viruses were constructed with the WR strain of vaccinia. PBL were suspended in RPMI 1640 medium (Gibco), supplemented with 10% heat-inactivated normal human serum, and 1×10^5 in a final volume of 0.1 ml of medium placed into wells of round-bottomed 96-well plates. To each well was added 0.1 ml medium containing ultraviolet-light inactivated v-env5 (1×10^6 PFU ml⁻¹ before inactivation), or non-disrupted LAV ($1 \mu\text{g ml}^{-1}$, $\sim 1 \times 10^5$ tissue culture infective dose₅₀) that was purified by two cycles of sucrose gradient centrifugations from supernatants of LAV-infected CEM cells, an HLA DR-negative T-cell leukaemia line. Six days after stimulation, each well of PBL was labelled with $1 \mu\text{Ci } ^3\text{H-TdR}$ (³H-thymidine, NEN) for 6 h, the cells were collected and the c.p.m. ³H-TdR incorporated was determined by liquid scintillation counting. Values shown for c.p.m. ³H-TdR incorporated are the mean values of 4 replicate wells. The stimulation index was calculated by dividing the c.p.m. ³H-TdR incorporated into stimulated cells by the c.p.m. incorporated into non-stimulated cells.

Table 2 Expression of IL-2 receptors and lymphocyte antigens on PBL from vaccinated macaques after stimulation

Macaque No.	PBL stimulated with	Total IL-2 receptor ⁺ cells (%)	% IL-2 receptor ⁺ cells co-expressing		
			CD4	CD8	CD20 (Bp35)
74	v-env5	27.7	48.6	52.2	2
80	v-env5	25.6	62.4	40	NT
74	LAV	14.7	50	59	1.9
80	LAV	12	58	38	1.5
80	no virus	0.3	<0.3	<0.3	<0.3

PBL isolated from macaques 74 and 80, following immunization and boosting with the recombinant vaccinia virus v-env5, were cultured in medium alone or were stimulated for 6 days with v-env5 or LAV as described in Table 1. PBL were then examined by two-colour immunofluorescence for expression of IL-2 receptors, T-cell associated CD4 and CD8 antigens and B-cell associated CD20 (Bp35) antigen using monoclonal antibodies previously found to react with macaque PBL⁴³. The PBL were stained simultaneously with phycoerythrin-conjugated monoclonal antibody 2A3 (Becton-Dickinson) to IL-2R and fluorescein-conjugated monoclonal antibodies 1F5²¹ (Genetic Systems Corp.) to CD20, G10-1⁴⁴ (Genetic Systems Corp.) to CD8, or T4_a (Ortho Diagnostics) to CD4. Antibodies were used at saturating concentrations as previously determined by titrations on lymphocytes analysed by flow microfluorometry with a modified FACS IV sorter (Becton-Dickinson). Quantitative two-colour analysis was performed as previously described⁴⁵. The forward and right-angle scatter gates were set to include lymphoblasts and a substantial number of small lymphocytes. The values shown are those for the total per cent IL-2 receptor⁺ cells and for the per cent IL-2 receptor⁺ cells co-expressing CD4, CD8 or CD20 (Bp35).

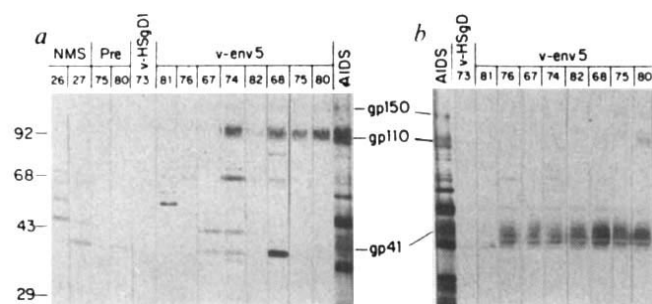


Fig. 1 Western blot analysis of serum samples from macaques immunized with a recombinant vaccinia virus expressing LAV envelope glycoproteins. Eight macaques of juvenile to adult age, were immunized intradermally at one site on the midline of the back. Four animals (68, 75, 80 and 82) received 2×10^8 PFU of the virus, and the other four (67, 76, 74 and 81) received 2×10^7 PFU. Twelve weeks after the primary immunization, all animals (except No. 81) received a second inoculation of 2×10^8 PFU. Four weeks later, serum samples were collected and analysed by Western blot. As negative controls, sera from two non-immunized macaques (26 and 27) two pre-immune macaques (pre 75 and 80) and one macaque (73) immunized with v-HSVgD1 were used. Serum from an HIV-seropositive individual (AIDS) was used as the positive control. LAV virion proteins resolved on a 7–15% SDS-polyacrylamide gel and immobilized on a nitrocellulose filter by electro-transfer. Strips from such filters were reacted with serum samples under conditions optimized for the detection of antibodies to either gp110 (a) or gp41 (panel b). a, Filter strips were reacted with serum samples diluted 50-fold in PBS plus 5% non-fat dry milk and 0.01% each of thimerosol and antifoam A. They were washed 5 times in PBS plus 0.05% Tween 20, once in PBS and then reacted with goat anti-human immunoglobulin antibodies conjugated with alkaline phosphatase. b, Serum samples were diluted (50-fold) in PBS plus 0.2% NP-40 and 3% non-fat dry milk. Filter strips were washed in PBS plus 0.2% NP-40 and then reacted with the same antibody-alkaline phosphatase conjugate as in (a). M_r markers show size in kilodaltons.

macaques immunized with v-env5 incorporated 2.5- to 14.9-fold more ^3H -thymidine 6 days following stimulation with LAV than did non-stimulated PBL (Table 1). PBL from all these macaques and also from macaque 73, which was immunized with the v-HSVgD1 recombinant virus, incorporated 12.8- to 91.9-fold more ^3H -thymidine than did non-stimulated PBL, following stimulation with v-env5 (Table 1) or with parental vaccinia virus (data not shown). However, PBL from non-immunized macaques 26 and 27 or from v-HSVgD1-immunized macaque 73 did not proliferate in response to stimulation with LAV on day 6 (Table 1) or on days 5 or 7 following stimulation with any of several concentrations of LAV (data not shown).

PBL from vaccinated macaques, stimulated for 6 days with LAV or v-env5, were examined by two-colour immunofluorescence for expression of IL-2 receptors that are present on activated T cells¹⁷ and activated B cells^{18,19}; CD4 and CD8 antigens present on T cells²⁰; and CD20 (Bp35) antigen present on B cells^{21,22}. Nearly all the virus-stimulated PBL that express IL-2 receptors co-express CD4 or CD8 antigens, whereas only 1.5–2% co-express CD20 (Bp35) antigen, indicating that v-env5- or LAV-activated PBL are T cells (Table 2).

It is well established that T cells, following antigenic stimulation, produce lymphokines (including IL-2) that can promote differentiation and/or proliferation of T and B cells^{23–27}, and can activate natural killer cells²⁸ that can lyse cells infected with several viruses including the AIDS virus^{29–31}. We therefore asked whether T cells from the vaccinated macaques produce IL-2 following stimulation with LAV. Supernatants from v-env5- or LAV-stimulated PBL from macaques immunized twice with v-env5 contained IL-2 (Table 3, exp. 1), shown by their ability to induce proliferation of CTLL-2 cells, an IL-2-dependent cell line³². Similarly, IL-2 was detected in supernatants of LAV or

Table 3 IL-2 production by PBL from vaccinated macaques after stimulation

Macaque No.	Immunization	IL-2 (U ml ⁻¹) in supernatants of PBL stimulated with:		
		No virus	LAV	Recombinant vaccinia v-env5
Exp. 1				
67	v-env5	0	28.4	96.0
68	v-env5	0	16.0	124.0
74	v-env5	0	9.0	52.0
73	v-HSVgDI	0	0	108
26	none	0	0	0
Exp. 2				
03	v-env5	0	14.4	44.8
05	v-env5 (N.Y.)	0	16.8	33.3
49	v-env5 (N.Y.)	0	7.1	26.7
52	v-env5 (N.Y.)	0	2.4	27.6
59	v-HSVgDI	0	0	24.2
26	none	0	0	0
27	none	0	0	0

PBL were isolated from heparinized blood of macaques 4 weeks following a second intradermal immunization with v-env5 or v-HSVgD1 constructed with the WR strain of vaccinia virus (exp. 1). For exp. 2, PBL were isolated from macaques 4 weeks after a primary intradermal immunization with 2×10^8 PFU of v-env5, v-HSVgD1 or a v-env5 recombinant virus constructed with v-env5 NY. PBL were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated normal human serum and 2×10^5 placed into wells of round-bottomed 96-well plates followed by the addition of ultraviolet-light inactivated v-env5 (1×10^6 PFU ml^{-1} before inactivation) or purified LAV ($1 \mu\text{g ml}^{-1}$) to the wells. Two days after stimulation, supernatants were collected from the replicate wells and were tested for their ability to support proliferation of IL-2-dependent CTLL-2 cells (kindly provided by Dr S. Gillis, Immunex Corp.) that were washed free of IL-2 for 24 h before the assay. During the last 6 h of incubation with the supernatants, the cells were labelled with ^3H -TdR and ^3H -TdR incorporated into the cells was determined. The units of IL-2 activity present in the supernatants were calculated as described³⁶ from standard curves obtained from testing the effect of recombinant human IL-2 (provided by Dr Gillis) on proliferation of CTLL-2 cells.

v-env5 stimulated PBL from macaque 03 (Table 3, exp. 2), which was immunized once with v-env5 and from all three macaques that were immunized once with a similar recombinant (v-env5 N.Y.) constructed using the New York City Department of Health strain of vaccinia virus that has been used as a smallpox vaccine in humans³³. In contrast, PBL from macaques 73 and 59, that were immunized with the vaccinia-HSV gD-1 recombinant virus, produce IL-2 only following stimulation with v-env5, and not after stimulation with LAV (Table 3). Non-immunized macaques 26 and 27 do not produce detectable IL-2 after stimulation with either LAV or the recombinant vaccinia virus. Because primarily T cells with helper/inducer activity produce IL-2 after antigenic stimulation^{23–26} these results demonstrate the presence of helper-T cells, which recognize LAV, in macaques immunized with the recombinant viruses. In addition to their probable role in the differentiation of B cells to produce antibodies to LAV envelope antigens, these IL-2-producing T cells may be involved in differentiation and/or expansion of effector cells, such as cytotoxic T lymphocytes or natural killer cells that can kill virus-infected cells.

In summary, our study demonstrates that immunization of macaques with recombinant vaccinia viruses expressing AIDS viral glycoproteins causes both the generation of antibodies and T-cell-mediated immune responses to the human AIDS virus. Because the envelope antigens are the only LAV antigens encoded by genes of these recombinant viruses, it is apparent that the T cells that proliferate and produce IL-2 following stimulation with LAV recognize the LAV envelope antigen(s). Studies are in progress to determine whether the T cells from

these vaccinated macaques recognize predominantly gp41 or gp110. Along these lines, we and others have recently found that vaccinia recombinant viruses expressing genes for herpes simplex and influenza viral antigens were useful for elucidating viral antigenic specificity of human T cells *in vitro*^{34,35}. Furthermore, immunization of rodents with a recombinant vaccinia virus expressing an influenza viral antigen-induced T-cell immunity *in vivo* and protection against influenza infections^{36,37}. Little is known about antigenic specificity of T cells to retroviruses. However an envelope glycoprotein, gp70, of Friend and Molony murine leukaemia virus (M-MuLV) has been reported to be recognized by M-MuLV immune mouse T cells^{38,39}. T lymphocytes directed against cells infected with human T-lymphotropic virus type I have been demonstrated⁴⁰, but the antigens that are recognized have not been reported.

Results presented here are the first demonstrating T-cell responses to AIDS envelope virus antigens and also demonstrating that vaccination of primates with a recombinant vaccinia virus that expresses a foreign viral antigen elicits a T-cell response to that antigen. We have not been able to determine whether cytotoxic T lymphocytes (CTL) to AIDS virus are also primed in the vaccinated macaques for the following reasons. First, an insufficient amount of blood can be obtained from these small monkeys for generating and maintaining CTL clones to test for lysis of v-env5 infected, but not parental vaccinia infected, target cells. Second, the lymphocytes of the macaques are relatively resistant to infection with AIDS virus and would not be suitable for target cells. However, we have begun experiments in chimpanzees, which are susceptible to infection with the human AIDS virus^{41,42} to determine whether vaccination with recombinant vaccinia viruses expressing LAV envelope glycoproteins will induce both helper and cytotoxic T-cell responses to LAV and also confer protection against infection with the AIDS virus.

Note added in proof: Results of preliminary experiments with Dr Jorg Eichberg (Southwest Foundation for Biomedical Research) indicate that T cells with both helper and cytotoxic activities directed against LAV envelope glycoproteins are present in chimpanzees immunized with a recombinant vaccinia virus expressing LAV envelope glycoproteins.

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Isolation of complementary DNA clones encoding the human lymphocyte glycoprotein T1/Leu-1

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The T1/Leu-1/CD5 molecule, a human T-cell surface glycoprotein of relative molecular mass (M_r) 67,000¹⁻³, has been implicated in the proliferative response of activated T cells⁴ and in T-cell helper function⁵. A similar involvement in T-cell proliferation has been reported for Ly-1 (refs 6-8), the murine homologue of T1 (ref. 9). Here we report the complete amino-acid sequence of the T1 precursor molecule deduced from complementary DNA clones. The protein contains a classical signal peptide; a 347-amino-acid extracellular segment; a transmembrane region; and a 93-amino-acid intracellular segment. The extracellular segment contains many cysteine residues and is composed of two related structural domains separated by a proline/threonine-rich region. The T1 molecule has structural features characteristic of other receptor molecules.

We purified the T1 molecule from the lymphoblastoid tumour cell line HPB-ALL using lectin and immunoaffinity chromatographies (Fig. 1a). The purified protein had a lower M_r (~56,000, Fig. 1a) than that previously described for the T1 molecule (67,000; refs 1, 2). But when the same immunoaffinity column was used to purify the T1 molecules in small scale from surface radioiodinated cells, we obtained a band of M_r 67,000 (Fig. 1b, lane 1). When both molecules were treated with endoglycosidase F (Endo F), removing all N-linked oligosaccharides¹⁰, there are identical shifts in mobility on SDS-polyacrylamide gels (Fig. 1b, lanes 2, 4). When the M_r 67,000 material was compared with the M_r 56,000 material by peptide mapping (Fig. 1c), we found extensive homology, indicating that the M_r 56,000 form is derived from the M_r 67,000 molecule.

It is likely that the M_r 56,000 T1 results from proteolysis during the isolation procedure because protease inhibitors were not added until after membranes were made from frozen cells. In contrast, radioiodinated viable cells from which the M_r 67,000 T1 was isolated were lysed and solubilized in the presence of protease inhibitors. When these cells are lysed in the absence of protease inhibitors some of the M_r 67,000 material is cleaved

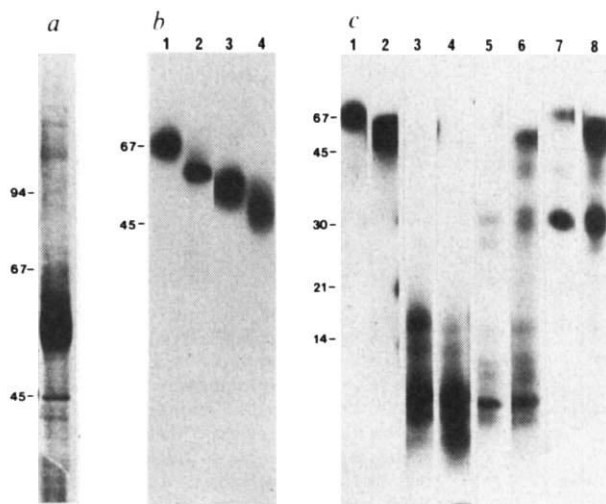


Fig. 1 Purification and analysis of T1. *a*, Silver-stained SDS-polyacrylamide gel of purified T1 (0.1 µg). *b*, SDS-polyacrylamide gel electrophoresis of Endo F cleavage products of 125 I-labelled T1. Lane 1, control M_r 67,000 T1; 2, endo F-digested M_r 67,000 T1; 3, control M_r 56,000 T1; 4, endo F-digested M_r 56,000 T1. *c*, One-dimensional peptide mapping of 125 I-labelled T1 using 10 µg of papain (lanes 3, 4), chymotrypsin (lanes 5, 6) or V8 protease (lanes 7, 8). Lanes 3, 5 and 7, M_r 67,000 T1; lanes 4, 6 and 8, M_r 56,000 T1. Undigested M_r 67,000 T1 and 56,000 T1 are in lanes 1 and 2, respectively.

Methods. *a*, Frozen HPB-ALL cells (5×10^{10} cells) were thawed, hand homogenized in a Potter Elvehjem homogenizer and crude membranes prepared by hypotonic lysis and differential centrifugation as described²⁵. The membranes were solubilized overnight at 4 °C in 500 ml extraction buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet P-40 (NP-40), 1 mM MnCl₂, 1 mM phenylmethylsulphonyl fluoride, 1 mM CaCl₂, 1 U ml⁻¹ aprotinin and 1 mM iodoacetamide) and centrifuged for 60 min at 100,000g. The supernatant was applied sequentially to lentil, ricin and wheat-germ lectin affinity columns (EY laboratories) which were eluted with lysis buffer containing 3% (w/v) α -methylmannoside, 10% (w/v) galactose or 5% (w/v) *N*-acetylglucosamine, respectively. Sodium deoxycholate (DOC) was added to the pooled lectin column eluates (0.5% final concentration) and applied to a column of anti-T1 monoclonal antibody 10.2 coupled to cyanogen bromide-activated Sepharose CL-4B (Pharmacia). The column was washed with two column volumes of the following buffers: (1) extraction buffer; (2) 0.5 M LiCl, 10 mM Tris, pH 7.5, 0.5% NP-40; (3) 10 mM Tris, 0.1% DOC; and eluted with 50 mM diethylamine, pH 11.5, 0.1% DOC. The eluted protein was precipitated with 5 volumes of cold acetone, resuspended in 500 µl water and 1 µl analysed on SDS-polyacrylamide gels that were silver stained to access yield and purity²⁶. Yield = 50 µg. *b*, Purified T1 protein (56,000 M_r) (3 µg) was 125 I-labelled with Iodobeads (Pierce) according to manufacturers instructions. HPB-ALL cells (5×10^7) were surface-iodinated with lactoperoxidase as described²⁷, solubilized with 1 ml extraction buffer and T1 protein (M_r 67,000) was purified on an anti-T1 antibody column as described above. An aliquot of each 125 I-labelled T1 preparation (with 10 µg soybean trypsin inhibitor as carrier) was acetone-precipitated and the pellet resuspended in 50 µl 50 mM EDTA, 0.5% NP-40, 0.1 M Na phosphate, pH 6.1, 1% 2-mercaptoethanol. Endo F (NEN) (1 U) was added to half of each sample and all samples were incubated 4 h at 37 °C. Samples were then analysed by SDS-polyacrylamide gel electrophoresis. *c*, An aliquot of each 125 I-labelled T1 preparation was acetone-precipitated as above and one-dimensional peptide mapping was performed on the soluble samples and analysed on a 7–19% gradient SDS-polyacrylamide gel as described²⁸.

to a band of a M_r ~56,000 (data not shown). This proteolytic cleavage is highly specific as only a single band of M_r 56,000 is seen and because other molecules purified from the same membrane preparations including HLA-A, -B, -C antigens; T-cell receptor α - and β - chains; T6 molecules; and T8 molecules are not degraded.

The 34 N-terminal amino acids of the M_r 56,000 T1 molecule were determined by sequencing the purified molecules on a gas-phase sequencer¹¹. The sequence H₂N-Arg-Leu-Ser-Trp-Tyr-Asp-Pro-Asp-Phe-Gln-Ala-Arg-Leu-Thr-Arg-Ser-Asn-Ser-Lys-X-Gln-Gly-Gln-Leu-Glu-Val-Tyr-Leu-Lys-Asp-Gly-Trp-X-Met was obtained twice. We synthesized two sets of non-overlapping oligonucleotide probes consisting of a 23mer of 128-fold degeneracy corresponding to amino acids 4–11 and a 17mer of 256-fold degeneracy corresponding to amino acids 21–26.

A cDNA library was constructed in the phage λ gt10 using

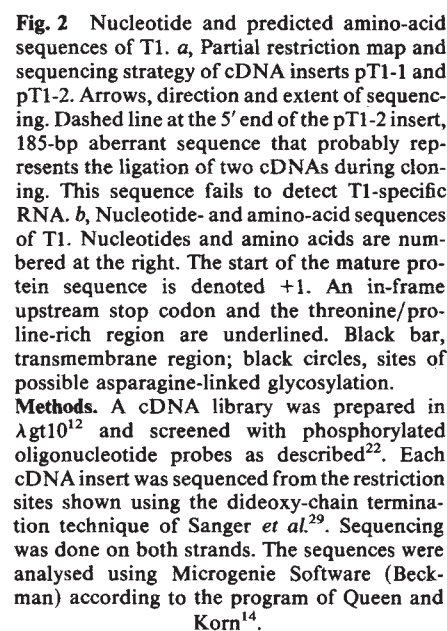
poly(A)⁺RNA from the human tumour T-cell line HPB-MLT as previously described¹², and we obtained one clone that hybridized with both probes. This clone, pT1-1, has an insert of ~1.0 kilobase (kb). A second clone of 2.1 kb, pT1-2, was obtained from the same cDNA library using the insert of pT1-1 as a hybridization probe. It overlaps the 3' end of the pT1-1 cDNA insert by 637 nucleotides, but does not contain the 5' sequence to which either of the oligonucleotide probes would hybridize.

The partial restriction maps for each insert (Fig. 2a) and the 2,320-base pair (bp) combined nucleotide sequences of both (Fig. 2b) are shown. The combined nucleotide sequence has a long open reading frame that contains a stretch of nucleotides starting at base 145 that corresponds to the N-terminal amino-acid sequence obtained from the purified T1 protein. There are three potential translation initiation ATG triplets at amino acid positions -8, -22 and -24 (bases 121–123, 79–81 and 73–75, respectively). The initiation site was tentatively placed at nucleotides 73–75 because this is the first ATG triplet that appears downstream from an in-frame termination codon TGA (nucleotides 49–51), and as it is flanked by nucleotides that fulfill criteria for a translation initiation site¹³. The open reading frame ends with the termination codon TAA at nucleotides 1,558–1,560. The 3' untranslated region is not complete as neither a poly(A) tail nor a polyadenylation signal (AATAAA) is present.

To determine the size of the messenger RNA encoding the T1 molecule, poly(A)⁺RNA from the HPB-ALL cell line was analysed on Northern blots using the nick-translated insert from pT1-1 (Fig. 3a, lane 1). Two mRNAs were detected at 3.6 and 2.7 kb. Thus, the 2,320-bp nucleotide sequence presented here lacks at least 400 bp. An identical hybridization pattern is seen when the insert from pT1-2 is used as a probe (data not shown). No hybridization is seen with mRNA from the T1-negative B lymphoblastoid cell line JY (Fig. 3a, lane 2).

The predicted amino-acid sequence (Fig. 2b) demonstrates several features of the T1 glycoprotein. The mature protein consists of 471 amino-acid residues with M_r 52,167. The 23 amino-acid residues between the initiation codon at residue -24 and the Arg residue at position 1 are characteristic of a classic signal sequence: they are hydrophobic, of the appropriate length and the sequence terminates at position -1 with a residue containing a small side chain (glycine). The presence of this signal sequence next to the experimentally determined N-terminal amino-acid sequence indicates the M_r 56,000 T1 probably resulted from a proteolytic cleavage from the C-terminus of the intact T1 molecule, possibly near a cluster of Arg and Lys residues at residues 379–391. A second hydrophobic region at amino acid residues 348–378 represents a putative 31-amino acid transmembrane region. To the C-terminal side of residue 378 there is a stretch of basic residues that is typical of sequences found on the cytoplasmic side of a transmembrane region.

There are 5 possible sites for N-linked glycosylation, 2 N-terminal to the transmembrane region and 3 C-terminal. Only the 2 N-terminal sites are used, as the purified M_r 56,000 T1 molecule, which contains the same number of glycans as the M_r 67,000 molecule (Fig. 1b), probably lacks most of the region C-terminal to the transmembrane region. As there is no precedent for glycosylation occurring on cytoplasmic residues, it is likely that residues 1–347 are extracellular and residues 379–471 are intracellular. The extracellular region is relatively cysteine-rich (22 cysteines) and contains a 20-residue stretch with 17 threonines and prolines (amino acids 114–133). The Thr/Pro-rich region forms an extended peptide that separates two homologous domains. Alignment of amino-acid residues 1–113 from the first domain with residues 242–347 from the second domain reveals a significant 30% amino-acid homology when alignment is optimized by proposing a single 6 amino-acid deletion between residues 290 and 291 (Fig. 4a). This strongly suggests that the domains arose by a gene duplication event. A model of T1 structure is shown in Fig. 4b. The nucleotide and



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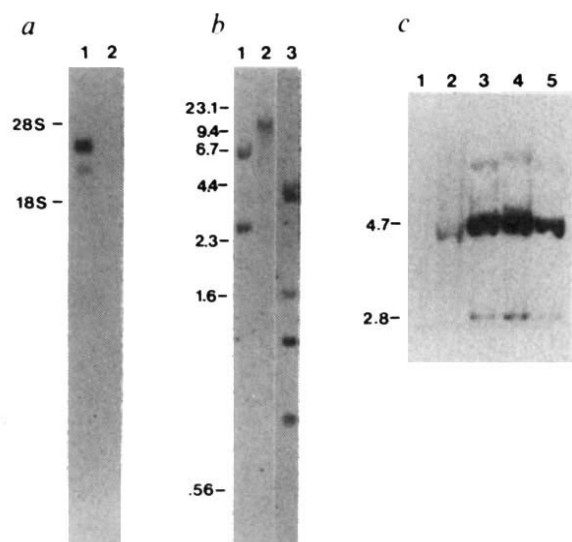


Fig. 3 *a*, Northern blot analysis of T1 mRNA. Poly (A)⁺ mRNA (2 µg) from the T cell line HPB-ALL (lane 1) or the B lymphoblastoid cell line JY (lane 2) was electrophoresed through a 1% agarose/formaldehyde gel and transferred to nitrocellulose as described³⁰. The filter was hybridized overnight with nick-translated pT1-1 insert (specific activity 10⁸ c.p.m. µg⁻¹) and washed as described³⁰. Sizes estimated from HPB-ALL 28S (5.2 kb) and 18S (2.0 kb) ribosomal RNAs. *b*, Southern blot analysis of T1 genomic DNA. HPB-ALL cellular DNA (10 µg) was digested with *Bgl*III (lane 1), *Eco*R1 (lane 2) or *Pst*I (lane 3) and separated through a 1% agarose gel. The DNA in the gel was denatured, blotted onto nitrocellulose and the filter hybridized overnight with nick-translated pT1-2 insert³⁰. Size markers, λ phage DNA digested with *Hind*III. *c*, Southern blot analysis of T1-amplified L cell transfectants. DNA (15 µg) from murine L cells (lane 1) or the T-cell line JM (lane 2) and DNA (5 µg) from three independent T1-L cell transfectants (lanes 3-5) were digested with *Hind*III, electrophoresed on an 0.8% agarose gel and analysed by Southern blots using the nick-translated insert of pT1-1 as probe^{16,30}. L cells were co-transfected with the herpes simplex thymidine kinase gene and total human DNA from the T-cell JM and T1-positive transfectants were selected using FACS³¹. Secondary transfectants were obtained by transfecting the DNA from three different T1 positive primary transfectants into L cells. These cells were amplified for T1 expression by several rounds of sorting the most positive 0.5% of cells as described¹⁷.

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Non-enzymatic cleavage and ligation of RNAs complementary to a plant virus satellite RNA

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Small satellite RNAs¹⁻⁵ of plant viruses depend on the presence of a supporting RNA virus for their propagation *in vivo*. Replication of the 359-nucleotide-long⁶ satellite RNA of tobacco ringspot virus (STobRV RNA) is detected only in tissue infected with tobacco ringspot virus (TobRV). STobRV RNA becomes encapsidated in TobRV coat protein and acts as a parasite of TobRV, reducing its accumulation and the severity of symptoms that it induces. Here we report the transcription *in vitro* of a circularly permuted, complementary DNA clone of STobRV RNA oriented so as to produce RNA that is complementary to the encapsidated, (+) polarity STobRV RNA. Like STobRV (+)RNA⁷, this dimeric, circularly permuted STobRV (-)RNA cleaves autolytically. Cleavage is at two identical sites generating monomeric-length RNA and two terminal fragments. The new termini are 5'-hydroxyl and 2':3'-cyclic phosphodiester groups. The RNAs ligate spontaneously to give linear and, from the monomers, circular molecules. Replication of STobRV RNA may require these autolysis and ligation reactions, which, at least *in vitro*, occur without enzyme catalysis.

Plasmid pSP641 (Fig. 1a) bears a copy of a budblight strain STobRV RNA sequence⁶ circularly permuted about residues 243 and 244 and in reverse orientation with respect to the bacteriophage SP6 RNA polymerase⁸ promoter. *In vitro* transcription⁹ of pSP641 should generate a slightly larger than dimer-sized STobRV (-)RNA, complementary to STobRV (+)RNA. However, RNA with the expected mobility of the

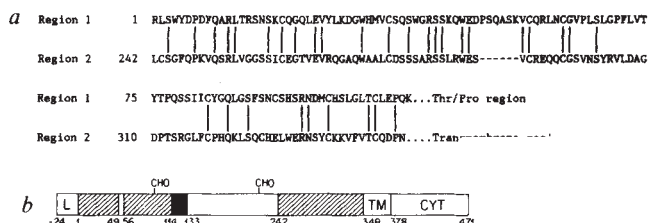


Fig. 4 *a*, Amino-acid sequence alignment of two homologous regions of T1. Region 1 spans residues 1-113 and region 2 residues 242-347. Dashes, 6-amino-acid deletion in region 2. *b*, Model of T1 structure. L, Leader or signal peptide; TM, transmembrane region; CYT, cytoplasmic region; CHO, positions of putative N-linked glycosylation; hatched regions, the two homologous domains.

monoclonal antibody; Dr David Andrews and William Lane for N-terminal amino-acid sequencing and oligonucleotide synthesis; Mary-Ann Lane for use of DNA-analysis program; and Dr James Woody (Naval Medical Research Institute) for providing HPB-ALL cells for protein purification. This work was supported by NIH AI15669 (J.L.S.), GM-17367, GM-28428 (L.A.H.), NCI 5R32 CA09031 (N.H.J.), CA04681 (H.-J.S.H.) and Damon Runyon-Walter Winchell Cancer Fund (D.P.D.).

Fig. 1 Structure of plasmid pSP641 and the autolytic processing of its transcripts. *a*, In plasmid pSP641 the bacteriophage SP6 promoter [XXX] is oriented for synthesis of STobRV (–)RNA (the RNA complementary to the STobRV (+)RNA encapsidated in TobRV coat protein during infection). Hatching, the complete, unpermuted STobRV monomeric (+)RNA sequence that is contained in the cloned, circularly permuted dimeric DNA copy of STobRV RNA. Numbers, nucleotide residues according to the STobRV (+)RNA sequence⁶; J(+), sites of the two junctions at which autolytic processing of the STobRV (+) RNA occurs⁷. Autolytic cleavage of STobRV (–)RNA is at the J(–) sites, located 48 residues from the J(+) sites. *b*, The expected primary transcript (P–M–D) of *Sma*I-linearized pSP641 and the products of its autolytic processing at one or both J(–). The autolytic reaction products, in 3' to 5' orientation and in order of increasing size are; the promoter distal fragment D, the promoter proximal fragment P, STobRV (–)RNA of monomeric length M, and the single-junction-containing RNAs P–M and M–D. The 3'-hydroxyl, 5'-hydroxyl, 2':3'-cyclic phosphodiester and 5'-triphosphate terminal groups are indicated. All the RNAs, except M, contain sequences derived from the vector (indicated by narrow lines at the 3' and 5' ends). The short line below the D fragment RNA defines a sequence to which a primer (Fig. 4) is complementary. *c*, Products of the *in vitro* transcription reaction with linearized pSP641 template were analysed by gel electrophoresis and autoradiography. With the exception of P–D and cM the detected zones are labelled according to *b*. P–D and cM were identified by analyses described in Figs 2, 4 and 5. The upper and lower marks to the left of the autoradiogram indicate the mobilities of dimeric and monomeric STobRV (+)RNAs, respectively. The mobilities of the zones designated by letters, except cM, were a logarithmic function of the number of nucleotide residues predicted. The origin of electrophoresis is indicated by O.

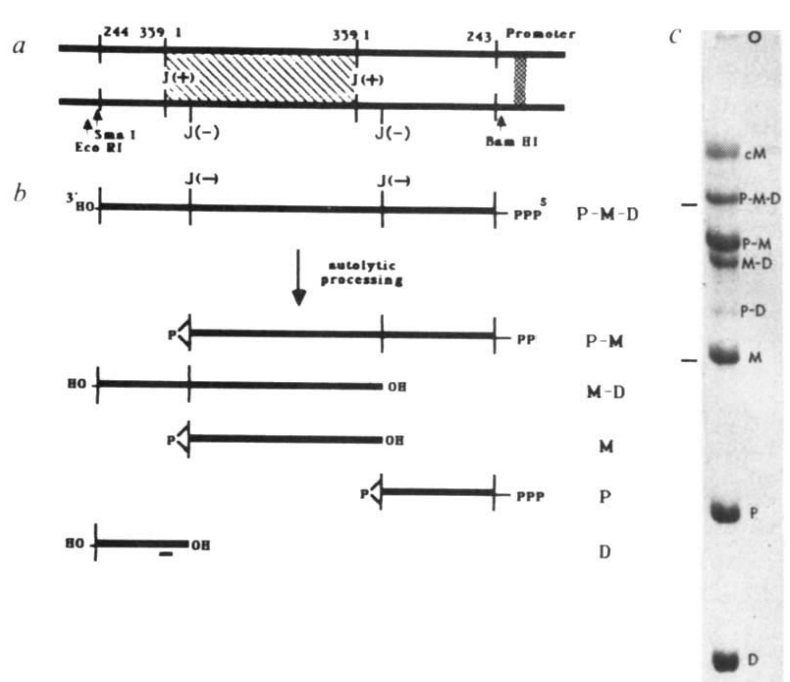
Methods. The fragment that was released by treating plasmid pBB1¹⁷ with restriction endonucleases *Eco*RI and *Bam*HI contains a circularly permuted dimeric copy of the budblight strain STobRV RNA sequence. It was ligated into similarly cut pSP64 (Promega Biotech) before transformation and colony selection to produce pSP641. The resulting plasmid pSP641 (10 µg) was linearized by treatment with restriction endonuclease *Sma*I. The DNA recovered after phenol/chloroform extraction and precipitation by ethanol was incubated at 40 °C for 1 h in 200 µl 40 mM Tris-HCl, pH 7.5, 20 mM NaCl, 6 mM MgCl₂, 2 mM spermidine hydrochloride, 10 mM DTT, 500 µM each rNTP, including 150 µCi [α -³²P]GTP, 200 U ribonuclease inhibitor, RNasin (Promega Biotech), and 200 U bacteriophage SP6 RNA polymerase (NEN). Additions of 100 µl each of water saturated phenol and chloroform terminated the reaction. After extractions and subsequent precipitation, RNAs were resolved by electrophoresis through 6.5% polyacrylamide gel in TBE buffer¹⁸ (90 mM Tris, 90 mM boric acid, 2.5 mM Na₂EDTA) and 7 M urea.

primary transcript, designated P–M–D in Fig. 1*b,c*, is only a minor reaction product. The more abundant RNAs are more rapidly migrating but probably do not arise by initiation within the STobRV RNA sequence because pSP641 template cut next to the promoter with *Bam*HI (Fig. 1*a*), gives no transcript.

We designate the three most rapidly migrating RNAs (Fig. 1*b,c*) according to the location of their sequences within the primary transcript: proximal (P) to the promoter; distal (D) to the promoter; and central (M) the full monomeric sequence. Electrophoretically purified P, M and D were separately incubated with bacteriophage T4 RNA ligase and [γ -³²P]pCp; electrophoresis revealed that only D accepted the radioactive label. When pSP641 was cleaved with *Eco*RI (Fig. 1*a*) rather than *Sma*I, increasing the expected size of the primary transcript by 15 nucleotides, the D fragment, but not M or P, had a slightly reduced mobility. We conclude that D is terminated by a 3'-hydroxyl group and is derived from the run-off-terminated 3' end of the primary transcript.

Of the RNAs P, M and D, only P was labelled by incorporation of [γ -³²P]GTP, confirming the location of P. Polynucleotide kinase catalysed the phosphorylation from [γ -³²P]ATP of M and D but not P (Fig. 2*a*, lane 1). pG was the only radioactive product identified by ion exchange thin layer chromatography (Fig. 2*b*) after digestion of terminally labelled M and D by nuclease P₁. We recovered P, M and D from a transcription reaction mixture which included [α -³²P]GTP. The only radioactive phosphate, in RNA fragments P (Fig. 2*c*) and M, that was resistant to nuclease P₁ and to calf intestinal alkaline phosphatase was, after alkaline hydrolysis, in Ap. No radioactive ribonucleotide phosphate (Np) was obtained from D. Thus both of the cleavage sites, J(–), in the primary transcript have an ApG bond.

The observed sizes of P and D predict that each J(–) site in the primary transcript is near nucleotide 50 (Fig. 1*b*). We assign the J(–) junction to residues 49–48 rather than to either of the two ApG bonds (residues 21–20 and 79–78) in this region of STobRV (–)RNA⁶. Partial nucleotide sequence determination¹⁰



of 5'-³²P-phosphorylated M and D confirms this assignment. Figure 3 compares the nucleotide sequences that are near J(–) and near J(+), the autolytic junction of STobRV (+)RNA.

RNAs P–M and M–D (Fig. 1*c*) were tentatively identified on the basis of their mobilities. P–M and P–M–D, in addition to P, were labelled by incorporation of [γ -³²P]GTP. [γ -³²P]GTP did not label M–D, but M–D was phosphorylated by polynucleotide kinase without prior phosphatase treatment (Fig. 2*a*, lane 1). In other experiments, electrophoretically purified P–M–D efficiently generated P, M and D when incubated under the conditions of the SP6 RNA polymerase reaction (Fig. 1 legend) but without added proteins, dithiothreitol (DTT) or ribonucleotide triphosphates (rNTPs). Similarly, purified P–M generated P and M and M–D generated M and D, showing the reaction at each J(–) junction to be non-enzymatic.

No autolysis product of P–M–D has a mobility corresponding to that expected for RNAs P–D, the linked promoter proximal and distal fragments, and cM, a circular form of the monomeric STobRV (–)RNA (Fig. 1*c*). We tested the possibility that P–D and cM arise by reversal of the autolytic reaction. The 233-nucleotide-long P, joined to the 167-nucleotide-long D, should create a 400-nucleotide-long RNA P–D that includes vector-derived, non-STobRV RNA residues; 34 at the 5' end and 2 at the 3' end. P–D resisted dissociation under the denaturing conditions of gel electrophoresis and served as a template for reverse transcriptase during extension of a synthetic oligodeoxyribonucleotide primer. The primed DNA transcript of P–D was of the expected size (zone 2, Fig. 4), implying that transcription proceeded through the junction J(–).

When incubated in a buffered solution of magnesium ions and spermidine, electrophoretically purified M generated two new species (Fig. 5*a*, lane 1). One has the mobility of dimeric STobRV (+)RNA and is designated M–M. The other, cM, the most slowly migrating species, was formed in greater yield (Fig. 5*a*). That cM is circular is indicated by several observations. Unlike M–M, cM was not formed from 5'-phosphorylated monomer incubated with an excess of unphosphorylated

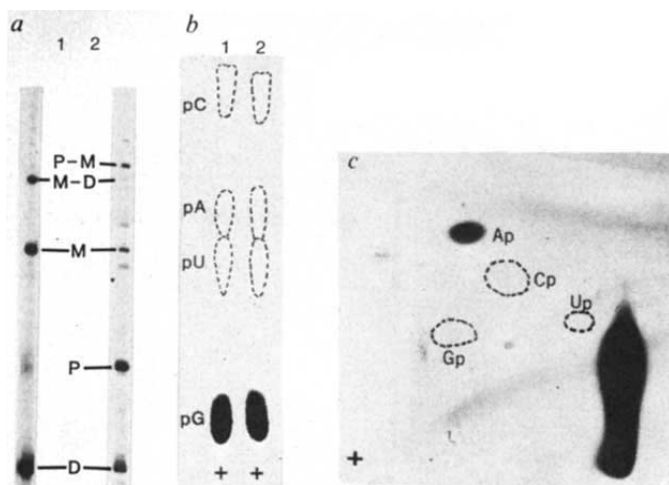


Fig. 2 Terminal residues formed by autolysis of P-M-D. Plasmid pSP641 was linearized with restriction endonuclease *Sma*I and transcribed as described in Fig. 1 except that no labelled rNTP was added. Part of the RNA recovered after phenol extraction and ethanol precipitation was incubated with calf intestinal alkaline phosphatase and purified. Both RNA samples were incubated with polynucleotide kinase and [γ - 32 P]ATP⁶ and analysed by electrophoresis as in Fig. 1. *a*, D, M and M-D accepted a phosphoryl group without prior phosphatase treatment (lane 1). Prior phosphatase treatment was required to achieve significant 5'-phosphorylation of P and P-M (lane 2). *b*, Modified RNAs [γ - 32 P]-M (lane 1) and [γ - 32 P]-D (lane 2) were recovered by soaking gel pieces¹⁰. Two micrograms of *Escherichia coli* transfer RNA (tRNA) plus [γ - 32 P]-M or -D were incubated with 0.36 units of nuclease P₁ at 37 °C for 20 min in 8 μ l 2:1:330 pyridine:acetic acid:water. Reaction products were mixed with 10 μ g of pN standards and were chromatographed on a 20-cm polyethylenimine cellulose thin layer in 5:1:32 acetic acid:pyridine:water to identify pG as the 5'-terminal residue of the phosphorylated RNA. *c*, Plasmid pSP641 was transcribed in a solution that contained [α - 32 P]GTP, as described in Fig. 1 legend. RNA from the P zone was digested with nuclease P₁ and the solution was vacuum-dried. This was digested with 0.001 IU calf intestinal alkaline phosphatase (Sigma) for 40 min at 37 °C in 10 μ l of 100 mM NH₄HCO₃, 34 mM NH₃. The vacuum-dried product was dissolved in 40 μ l of 20% piperidine and incubated at 95 °C for 90 min to open the 2':3'-cyclic phosphodiester bond. Two-dimensional cellulose TLC and autoradiography⁶ revealed Ap and inorganic phosphate, indicating that 5'-phosphoryl-adenosine-2':3'-cyclic phosphodiester was present in the P₁ nuclease digest.

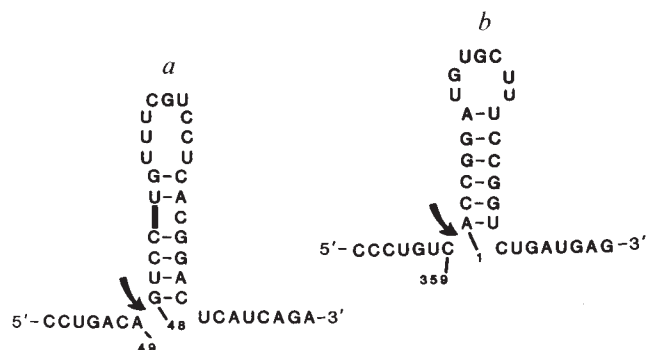


Fig. 3 Comparison of nucleotide sequences near the autolytic processing site of STobRV (-)RNA (*a*) with the sequences near the junction of STobRV (*b*). The two patterns of secondary structure were selected in an attempt to discover local similarities between the sites, but they are otherwise arbitrary and not supported by data presented here. Arrows indicate the bond that is cleaved in each sequence to form a new 5'-hydroxyl group and a new 2':3'-cyclic phosphodiester group.

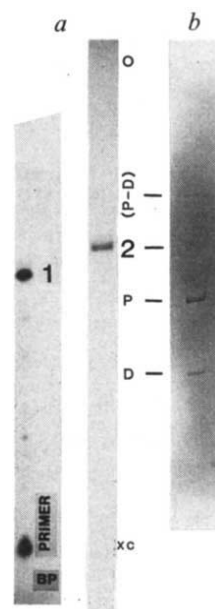


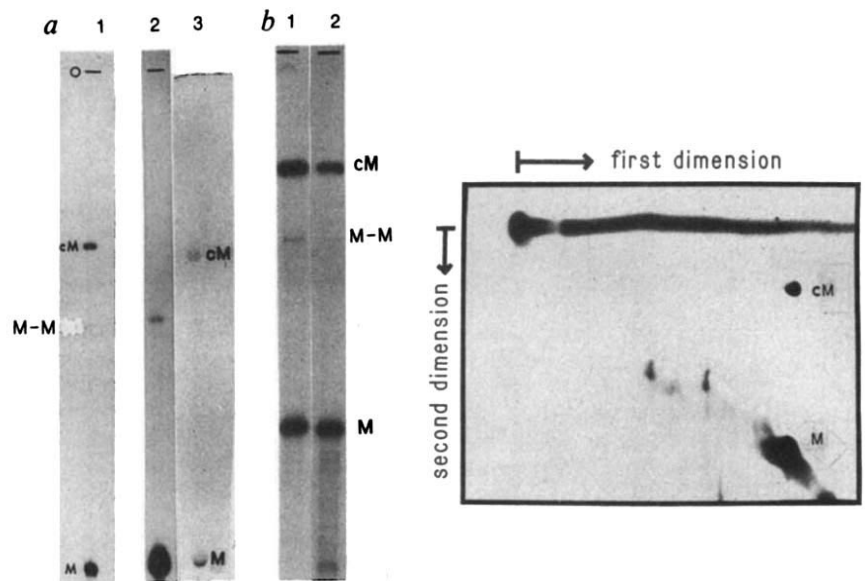
Fig. 4 Reverse transcription of spontaneously joined STobRV (-)RNA fragments. Unlabelled transcript fragments P and D were isolated, combined and incubated to form P-D. The mixture of P-D, P and D was the template for a reverse transcriptase-catalysed reaction using a primer that is complementary to D, as indicated in Fig. 1*b*. *a*, After electrophoresis autoradiography revealed zone 1 (39% of the radioactivity), which contained the 59 residue transcript of D and zone 2 (15% of the radioactivity) which contained the approximately 290 residue transcript of (P-D). The remaining 46% of the radioactivity was in the primer zone. *b*, Toluidine blue O staining¹⁹ located the P, D and P-D zones, and, more weakly, zone 2, but no zones were detected in the lower portion of the gel (not shown). Positions of the bottom of the gel well, O, and tracking dyes bromophenol blue (BP) and xylene cyanol (XC) are marked. Zone 1 but not zone 2 formed with D alone as template, whereas P gave no product (data not shown).

Methods. Electrophoretically purified P and D were mixed in a volume of 20 μ l, at a concentration of approximately 0.5 mg ml⁻¹ and incubated for 1 h at room temperature and pH 7.5 in 40 mM Tris-HCl, 20 mM NaCl, 6 mM MgCl₂, 2 mM spermidine hydrochloride, which are conditions suited to spontaneous ligation. The synthetic oligodeoxyribonucleotide dGATACCTGTCACCGGATGTGCTTTC (from the University of California, Davis Protein Structure Laboratory), which corresponds in sequence to residues 349-359 of the J(+) region of STobRV (+)RNA, was labelled by incubation with bacteriophage T4 polynucleotide kinase (NEN) and [γ - 32 P]ATP and was purified by electrophoresis through a 20% polyacrylamide gel in TBE and 7 M urea. The primer gel zone was soaked for 24 h at 37 °C with shaking in 350 μ l of 100 mM Tris, 84 mM HCl, 330 mM sodium acetate (pH ~7.5), and the gel removed. The solution and the reaction mixture containing P+D RNA fragments were mixed and heated to 90 °C for 2 min. The temperature was then reduced to 60 °C immediately and to 35 °C over a period of 2 h. The primer-template complexes were precipitated by addition of ethanol. The precipitate was dissolved in 50 μ l of solution pH 8.3: 66 mM Tris, 26 mM HCl, 50 mM KCl, 8 mM MgCl₂, 10 mM DTT, 100 μ g ml⁻¹ actinomycin D, 0.2 mM each dNTP, and 200 U each of RNasin and avian myeloblastosis virus reverse transcriptase (Life Sciences, St Petersburg, Florida). Incubation was for 5 min at room temperature, 5 min at 37 °C, and 40 min at 42 °C. The RNA/DNA hybrid was recovered by extraction of the solution with phenol and chloroform and precipitation by addition of sodium acetate and ethanol. Electrophoresis was through an 80 cm 6.5% polyacrylamide gel in TBE buffer and 7 M urea. The stained gel was photographed and autoradiographed. The primer gel zone and zones 1 and 2 were excised and Cerenkov counted.

monomer (Fig. 5*a*, lane 2) and could not be labelled in a polynucleotide kinase-catalysed reaction that readily labelled other satellite RNA species (methods see Fig. 2). cM failed to accept [γ - 32 P]pCp in an RNA ligase catalysed reaction even after phosphatase treatment. When cM was incubated in SP6 RNA polymerase buffer and subsequently analysed by gel electrophoresis, we observed M, cM and M-M zones in that order of decreasing abundance. Material from the M zone also produced a mixture of M, cM and M-M upon subsequent incubation, showing reversibility of the reaction.

Ribonuclease T₁ treatment of cM and M in urea solution did not generate a new zone migrating between the cM and M zones.

Fig. 5 Spontaneous circularization of monomeric RNA. Monomeric STobRV (–)RNA was electrophoretically purified from the mixture of RNAs that accumulated during *in vitro* transcription of *Sma*I-linearized pSP641 (Fig. 1). **a**, When incubated in buffered solution of MgCl₂ and spermidine hydrochloride, internally labelled monomeric STobRV (–)RNA, M, formed two other species, designated M–M and cM, that were resolved by electrophoresis through 6.5% polyacrylamide gel in 7 M urea. M–M had the mobility of dimeric STobRV (+)RNA. Unlabelled M from transcription of pSP641 was partially ³²P-5'-phosphorylated in a polynucleotide kinase catalysed reaction. On incubation this RNA produced a radioactive M–M zone and a cM zone that was detected by toluidine blue O staining¹⁵ (lane 3) but not by autoradiography (lane 2). Thus 5'-phosphorylated M did not form cM. **b**, Electrophoretically-purified, internally-labelled cM partially reverted to a mixture of cM and M during elution from the gel. The radioactive cM and M mixture was combined with fragmented, unlabelled P and D RNAs to a final concentration in the reaction mixture of 0.6 mg ml^{–1}. The mixed RNAs were incubated alone (lane 1) or with 1.0 unit ml^{–1} ribonuclease T₁ (lane 2) for 2 min at 55 °C in 6.0 M urea, 15 mM sodium citrate buffer, 19 mM EDTA, pH 5.0. Even an autoradiographic exposure 8 times longer than that shown gave no indication of zones between the cM and M zones, except for the minor M–M zone. Cerenkov radioactivity for the cM and M zones, respectively, were 1,183 and 1,076 c.p.m. (lane 1) and 471 and 894 c.p.m. (lane 2), thus indicating conversion of cM to M if cM and M are similarly susceptible to ribonuclease T₁. **c**, Zone cM migrated off the main diagonal of autoradiographically detected zones after two dimensional electrophoresis. The internally labelled cM and M were not resolved in 6% polyacrylamide gel in 10% glycerol (no urea; first dimension), but cM migrated more slowly than M in 8% polyacrylamide gel in 8 M urea (second dimension), which was confirmed by separate one dimensional electrophoretic analyses of the electrophoretically purified M and cM species.



This is as expected if cM is converted to a linear M-sized RNA by a single break in the polyribonucleotide chain. M and cM had the same mobility during electrophoresis in a gel under non-denaturing conditions, whereas cM had a much smaller mobility than M at elevated temperature in an 8 M urea denaturing gel (Fig. 5c). Circular and linear forms of other small RNAs exhibit similar differential mobilities^{11–13}. When primed and incubated with reverse transcriptase as described for P–D in Fig. 4, cM was also transcribed; most of the resulting cDNA remaining in and near the gel well.

We have shown that polyribonucleotides with STobRV (–)RNA sequences are capable of autolytic processing at a single site 48 residues distant from the site at which autolytic processing of STobRV (+)RNA occurs. The reverse of this reaction, the spontaneous ligation of STobRV (–)RNA to form linear or circular molecules, presumably requires an attack by the 5'-hydroxyl group of the terminal guanylate residue on the 2':3'-cyclic phosphodiester group of the terminal adenylate residue. How the RNA structure, stabilized by spermidine and/or magnesium ions, facilitates the cleavage and spontaneous ligation is unknown.

Monomeric STobRV (+)RNA from virus particles forms dimeric STobRV in a yield of a fraction of a per cent when incubated at a concentration of 1 mg ml^{–1} under nonphysiological conditions of 4 °C overnight in the presence of zinc ions⁷. The yield of non-enzymatically ligated STobRV (+)RNA is even less under the conditions of the SP6 RNA polymerase reaction or if less than the complete monomer polynucleotide chain is reacted (our unpublished observations). Thus the non-enzymatic ligation reaction of STobRV (–)RNA is far more efficient than the corresponding reaction for STobRV (+)RNA, and the two reactions may be qualitatively as well as quantitatively different.

We presume that the bulk of the multimeric STobRV (–)RNA that accumulates¹⁴ after mixed inoculation of plants with TobRV and STobRV RNA results from the rolling circle transcription of circular forms of STobRV (+)RNA. The multimeric (–)RNA so generated is clearly capable of specific autolytic cleavage to form monomeric STobRV (–)RNA. Under our *in vitro* conditions monomeric STobRV (–)RNA reacts more readily to form cM than dimers or higher-order, linear multimers, making it unlikely that spontaneous ligation reactions are the primary source of the observed¹⁴ STobRV (–)RNA multimers. Two possible origins of the multimeric forms of STobRV (+)RNA

are transcription of multimeric STobRV (–)RNA, as postulated previously¹⁴, or rolling circle transcription of circular STobRV (–)RNA templates¹⁵. We have shown that at least one polymerase, reverse transcriptase, is able to use the spontaneously ligated RNAs P–D and cM as its template.

In addition to STobRV RNA, two other small RNAs have been shown to undergo autolytic reactions of both complementary strands: avocado sunblotch viroid¹⁶ and lucerne transient streak RNA 2 (a satellite RNA; A. C. Forster, D. B. Mitchell, P. Keese and R. H. Symons, unpublished data). No non-enzymatic ligation reaction has been reported for these RNAs, and the processing sites in the two complementary RNAs are closer than J(–) and J(+) are in the STobRV RNA sequence. The existence of an autolytic reaction in both strands of a viroid and two satellite RNAs that have only very limited nucleotide sequence homology⁶ implies a general role for these reactions in the replication of small RNAs.

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A cyclic AMP- and phorbol ester-inducible DNA element

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Many cellular processes are regulated by hormones and neurotransmitters which interact with cell-surface receptors to produce intracellular second messengers that activate protein kinases. Cyclic (c) AMP is a second messenger whose intracellular level is determined by receptor-mediated activation or inhibition of adenylate cyclase^{1,2}. Phorbol esters directly activate protein kinase C, a Ca²⁺ and phospholipid-dependent protein kinase³ and a component of a different second messenger system, the phosphatidylinositol pathway⁴. Proenkephalin messenger RNA levels are regulated in response to cAMP analogues⁵, activators of adenylate cyclase⁶, nicotinic agonists⁷ and depolarization⁸, suggesting that expression of the gene encoding proenkephalin is regulated by trans-synaptic events involving cell-surface-receptor activation. Here we report that cAMP analogues and activators of adenylate cyclase regulate a proenkephalin-chloramphenicol acetyl transferase fusion gene when transiently expressed in tissue culture cells. Phorbol ester regulates the fusion gene in a similar fashion, but requires the presence of phosphodiesterase inhibitors for large effects. The DNA sequences required for regulation by both cAMP and phorbol ester map to the same 37-base pair (bp) region located 107-71 bp 5' to the mRNA cap site of the proenkephalin gene. This highly conserved region is composed of three closely related 12-bp sequences and has properties similar to those of previously characterized transcriptional enhancers.

To study the regulation of the proenkephalin (*enk*) gene^{9,10}, we constructed the plasmid pENKAT-12, containing human (h) proenkephalin control sequences fused to the bacterial chloramphenicol acetyl transferase¹¹ (*cat*) gene (Fig. 1a), a reporter gene whose expression can be easily monitored in tissue culture cells. After transfection of CV-1 cells with pENKAT-12, a fivefold increase in *cat* activity is observed when the cells are treated with the cAMP analogue 8-bromo-cAMP (8Br-cAMP) (Fig. 1b, lane 2) and an 18-fold increase is observed after treatment with 8Br-cAMP in the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methyl-xanthine (IMX) (Fig. 1b, lane 6). Cholera toxin (data not shown) and forskolin (Fig. 1b, lane 7) both stimulate adenylate cyclase and greatly stimulate pENKAT-12-directed *cat* activity. No induction of *cat* activity by cAMP is observed with constructions containing the herpes simplex virus (HSV) thymidine kinase (TK) promoter, pUT-KAT-3 (Fig. 2a, panel 6) or with the rous sarcoma virus (RSV) promoter (pRSVCAT, data not shown), indicating that the effect is relatively specific to the *henk* promoter. Although adding either IMX (Fig. 1b, lane 3) or the phorbol ester phorbol 12-myristate 13-acetate (TPA) (Fig. 1b, lane 4) alone has little stimulatory effect, treatment with 100 nM TPA in the presence of 0.5 mM IMX produces a 20-fold stimulation of *cat* activity (Fig. 1b, lane 5).

S₁ analysis demonstrates that RNA isolated from human pheochromocytoma (Fig. 1c, lanes 1, 2) and pENKAT-12-trans-

Table 1 5' Deletion analysis of cAMP regulation

5'-deletion endpoint	Control	<i>cat</i> activity 8Br-cAMP +IMX	Induction (times)
-193	1.0	6.0	6.0
-154	0.82	5.6	6.8
-119	0.53	3.5	6.6
-107	1.1	6.7	6.1
-97	0.74	2.8	2.9
-84	0.32	0.67	2.1
-72	0.43	0.44	1.0
-37	0.31	0.39	1.2
+154	0.11	0.12	1.1

A series of *Bal31* deletion mutants whose endpoints are indicated were assayed for their inducibility in the presence of 8Br-cAMP and IMX as described in Fig. 1. Relative *cat* activities for each deletion mutant in the presence and absence of 1.0 mM 8Br-cAMP+0.5 mM IMX were determined and inductions indicated in the right-hand column. The family of pENKAT-12 *Bal31* deletion mutants was generated by digestion of a plasmid containing the first 1,222 bp of the *henk* gene at the *EcoRI* site at position -193 followed by digestion with *Bal31* and insertion of *EcoRI* linkers before ligation. The *EcoRI* to *XbaI* fragments encompassing the *henk* promoter, exon 1 and intron A from this set of deletion mutants were then used to replace the equivalent region in pENKAT-12. The +154 deletion mutant was constructed by deletion of the *EcoRI* to *XbaI* fragment, removing all sequences between -193 and +154. The endpoints of each *Bal31* deletion mutant were determined by dideoxy-sequencing.

ected CV-1 cells (Fig. 1c, lane 4), but not control CV-1 cells (Fig. 1c, lane 3), protects a 70 nucleotide (nt) DNA fragment (marked *henk*) extending from the 5' end of the S₁ probe to the previously mapped *henk* cap site^{12,13}. Treatment of pENKAT-12-transfected CV-1 cells with 8Br-cAMP and IMX results in a level of induction of correctly initiated RNA transcripts (Fig. 1c, lane 5) in good agreement with the observed increase in *cat* activity.

To localize the sequence(s) required for cAMP and phorbol ester regulation we replaced all of the *henk* 3'-untranslated and flanking sequences of pENKAT-12 with HSV TK 3'-untranslated and flanking sequences. This plasmid responds to cAMP and phorbol esters in a fashion identical to pENKAT-12, indicating that *henk* 5'-untranslated and flanking sequences are sufficient to mediate regulation. To localize the regulatory sequences further, we constructed a 97-bp deletion in the *henk* 5' flanking DNA by digestion with the restriction enzyme *Bss*HII followed by re-ligation. The resulting plasmid, pENKAT-ΔB (Fig. 2a, panel 2), lacking the sequences between -155 and -58 bp 5' of the *henk* cap site, shows a fivefold reduction in basal *cat* activity and is not inducible with cAMP or TPA (Fig. 2a, compare panels 1 and 2). Reinsertion of the *Bss*HII fragment into pENKAT-ΔB in the opposite orientation restores both basal and regulated expression (Fig. 2a, panel 3).

The location of the regulatory region in the *henk* gene was independently mapped by generating a series of *Bal31* 5' deletions starting at the *EcoRI* site (Table 1). The induction drops ~twofold when the deletion extends into the region between -107 and -97 bp 5' to the cap site, whereas deletions extending to -72 completely remove cAMP and TPA regulation (Table 1). Basal expression drops slowly as deletions extend toward the TATA box and drops ~10-fold when the entire promoter region is deleted, observations similar to the results of Terao *et al.*¹³

To test if 5'-flanking sequences of the *henk* gene can confer cAMP and phorbol ester regulation on another promoter, we inserted DNA fragments from this region upstream of nucleotide -84 of a mouse proopiomelanocortin (*pomc*)-*cat* fusion gene, *pomcat*-84 (Fig. 2a, panel 4). The plasmid pPOMCAT-84 expresses basal *cat* activity at levels three- to fourfold lower than pENKAT-12, and shows only slight increases in *cat* activity

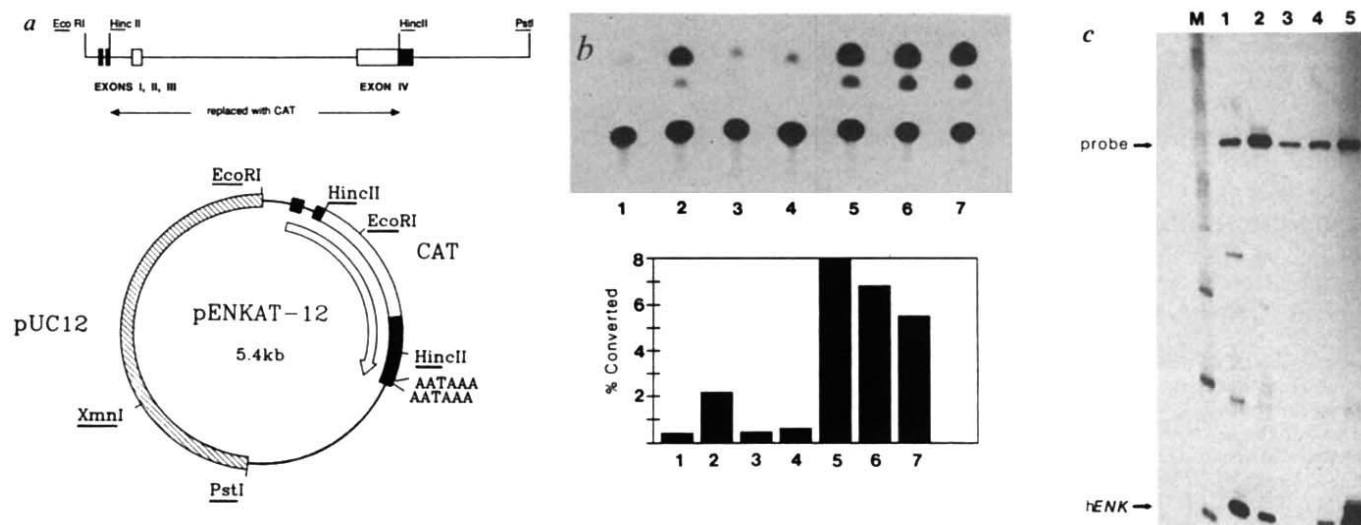


Fig. 1 Construction of a *henk*-*cat* gene and regulation by cyclic nucleotides and phorbol ester. *a*, Top, structure of the *henk* gene; below, protein-coding region of the *henk* gene (open box, bounded by two *HincII* sites) replaced by the *cat* coding region. Shaded boxes, untranslated regions. The resulting plasmid, pENKAT-12, is indicated in schematic form below. *b*, Induction of CAT enzyme activity in CV-1 cells following transfection with pENKAT-12 and treatment with lane 1, no regulator; 2, 1 mM 8Br-cAMP; 3, 0.5 mM IMX; 4, 200 nM TPA; 5, 200 nM TPA + 0.5 mM IMX; 6, 1 mM 8Br-cAMP + 0.5 mM IMX; 7, 25 μ M forskolin + 0.5 mM IMX. Regulator treatments were for 8 h starting 20 h after transfection. Upper panel, an autoradiogram of a TLC plate exposed for 2 h at -70° . Bars in the histogram in the lower panel, c.p.m. present in the acetylated forms of chloramphenicol (the upper two spots) in each lane expressed as a percentage of the total counts in chloramphenicol. *c*, S₁ analysis of *henk*-*cat* fusion RNA transcripts from transfected CV-1 cells. Lanes 1 and 2, RNA isolated from human pheochromocytoma; and from CV-1 cells transfected with the following: lane 3, no DNA; lanes 4, 5, pENKAT-12. Lane 5, RNA from CV-1 cells treated with 8Br-cAMP and IMX. M, radioactive ladder of self-ligated 34-bp oligonucleotide with the 68-bp dimer at bottom. RNA was analysed using a 5' end-labelled probe to region -193 to +70 of the *henk* gene. Correctly initiated transcripts from the *henk* promoter (70 bp) are indicated (arrow, marked *henk*). Lanes 1 and 2 contain 5 and 10 μ g pheochromocytoma RNA, respectively; all other lanes contain 30 μ g CV-1 RNA.

Methods. Construction of pENKAT-12 was carried out by stepwise cloning into pUC12 of fragments from both the *henk* gene and the *cat* region of pOCAT³¹. A 406-bp *EcoRI* to *HincII* *henk* gene² fragment containing 193 bp of 5' flanking sequence, exon I (70 bp), intron A (87 bp) and 53 out of 56 bp of exon II (all untranslated) was fused with the *cat* gene by insertion into the *EcoRI* to *SmaI* polylinker sites of pOCAT. We replaced pOCAT 3' control sequences with *henk* 3' control sequences by inserting a 1.2-kb *HincII* to *PstI* fragment containing 3' untranslated and flanking sequences of the *henk* gene downstream of the *cat* sequences to provide *henk* poly(A) addition signals. CV-1 cells were plated at a density of 4×10^5 per 10 cm Falcon tissue culture plate. Transfection of pENKAT-12 was performed by CaPO₄ precipitation³² 16–20 h after plating cells. In all experiments a plasmid carrying the β -galactosidase gene linked to the RSV promoter/enhancer (pRSV β gal³³) was co-transfected to provide an internal control for differences in transfection efficiency between different precipitates³³. pENKAT-12 (5 μ g) was cotransfected into CV-1 cells in the presence of 10 μ g pRSV β gal and 10 μ g pUC18 DNA. Regulators were added directly to culture media (1 $\times$ Dulbecco's modified Eagle's medium and 10% fetal calf serum) as 100 $\times$ stocks and TPA as a 5,000 $\times$ stock. Cells were collected by scraping with a rubber policeman in phosphate-buffered saline. Extracts were prepared by lysis of the cells in 100 μ l 0.25 M Tris-HCl (pH 7.5), 0.5% Triton X-100 followed by centrifugation at 15,000g for 10 min. Supernatants were removed and CAT activities measured. CAT enzyme reactions were performed as described¹¹ using 0.6 μ Ci [¹⁴C] chloramphenicol and 30 μ l cell extract at 37 $^{\circ}$ C for 2 h. Acetylated reaction products were resolved by TLC analysis using Baker-flex silica gel IB-F TLC plates and a 95% chloroform/5% methanol mobile phase. Following autoradiography the acetylated forms were excised and counted in a scintillation counter. The c.p.m. determined were then normalized to the β -galactosidase activities in each extract. In general, the β -galactosidase activities directed by the RSV promoter did not vary significantly in response to regulators. We have not detected effects of the internal control plasmid on CAT activity in any of the experiments reported. After exposure of CV-1 cells to DNA (24 h), RNA was isolated by guanidium thiocyanate extraction followed by centrifugation through CsCl. A single-stranded probe complementary to nucleotides -193 to +70 of the *henk* gene was prepared by primed DNA synthesis from pENKAT-12. RNA (30 μ g) isolated from transfected CV-1 cells or 5 and 10 μ g RNA isolated from human pheochromocytoma was mixed with 10 fmol end-labelled probe (6×10^5 c.p.m. fmol⁻¹) and S₁ analysis carried out as described by Berk and Sharp³⁴.

in response to regulators (Fig. 2a, compare panels 1 and 4). Fusion of the -193 to -80 *EcoRI*-*PstI* fragment of the *henk* gene to the *pomc* promoter at position -84 to generate pENK-POM-1, increases basal expression two- to fourfold, and confers >20-fold inducibility by regulators (Fig. 2a, panel 5). Insertion of *henk* sequences upstream of position -109 of the HSV-TK promoter results in a 50% increase in basal *cat* expression and confers two- to threefold inductions with regulators (Fig. 2b, panels 6, 7).

The ability of the -193 to -80 region of the *henk* gene to confer enhanced basal and regulated expression in an orientation independent manner and to confer regulated expression on two heterologous promoters (described above) indicates that this region shares properties with the viral enhancers^{14–17} and several other mammalian regulatory sequences^{18,19}. To test this, one or two copies of a 247-bp *PstI* fragment of bacteriophage λ DNA were inserted into the *PstI* site of pENK-POM-1 positioning the *henk* region either 247 or 494 bp upstream of the truncated mouse *pomc* promoter. A comparison of the *cat* activities of pENK-POM-1 alone (Fig. 2a, panel 5) to pENK-POM-1 with 247 or 494 bp phage λ DNA inserted into the *PstI* site (Fig. 2b, panels 2 and 3) shows that increasing the distance between the *henk* DNA and the *pomc* promoter decreases basal expression but does not substantially affect the large inductions

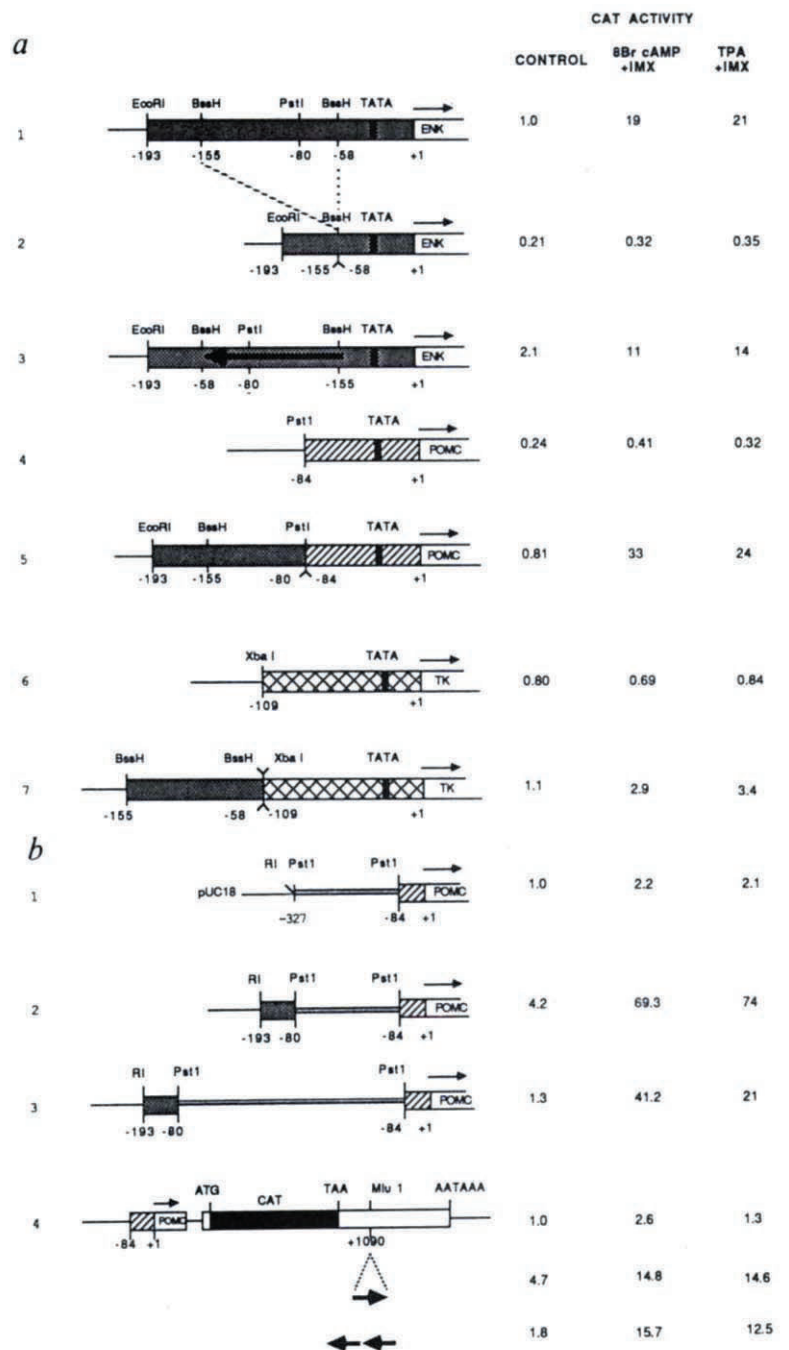
(>20 fold) of *cat* activity by cAMP or TPA. A control plasmid containing only λ spacer DNA (Fig. 2b, panel 1) shows similar basal *cat* activities and essentially no induction with regulators.

Either one copy in the normal or two copies in the inverted orientation of the *Bss*HII fragment of pENKAT-12 was also positioned 1.1 kilobases (kb) downstream of the cap site of pPOMCAT-84 (Fig. 2b, panel 4) between the termination codon of the *cat* gene and the polyadenylation signal of the *henk* gene. The construction containing two copies of the fragment showed significant inducibility with regulators although at a reduced level compared with pENK-POM-1 (compare Figs 2a panel 5 with 2b panel 4).

To define the regulatory sequences further we synthesized two double-stranded oligonucleotides that span the *henk* 5' flanking regions between -71 and -100 (oligo A) and between -101 to -133 (oligo B) (Fig. 3a). The two oligonucleotides, each with cohesive *PstI* termini, were introduced individually or in combination into the *PstI* site at -84 of pPOMCAT-84. Plasmids were identified that contain single or multiple copies of each oligonucleotide or the oligo A and B combination and their *cat* activities in the presence or absence of regulators were measured and compared with pPOMCAT-84 and pENK-POM-1. When inserted in single or multiple copies into the *PstI* site (-84) of pPOMCAT-84, oligo B cannot confer regulated expression to

Fig. 2 Localization and analysis of DNA sequences necessary for cAMP and phorbol ester regulation. *a*, Deletion and transfer analysis of *henk* 5'-flanking sequences. The -58 to -155 region was deleted in the *henk* promoter by *Bss*HII digestion of pENKAT-12 (panel 1, top) and religation to construct pENKAT-ΔB (panel 2). The purified 97-bp *Bss*HII fragment was cloned into pENKAT-ΔB in the opposite orientation (panel 3). A 109-bp *Eco*RI-*Pst*I fragment from the *henk* gene corresponding to positions -193 to -80 was fused to pPOMCAT-84 (a mouse *pomc*-*cat* fusion gene truncated to position -84) [panel 4], to create pENK-POM-1 (panel 5). The 97-bp *Bss*HII fragment from the *henk* gene was transferred to the promoter region of the *hsv-tk-cat* fusion gene, pUTKAT-3 (panel 6), upstream of position -109 (panel 7). *b*, Distance independence of regulation. The cyclic nucleotide and TPA inducible element defined by the *Eco*RI-*Pst*I region of *henk* 5'-flanking DNA to the mouse POMC promoter was moved 247 (panel 2) and 494 (panel 3) bp further upstream from the mouse POMC promoter by insertion of a 247-bp pair fragment of DNA contained within the bacteriophage λ *exo* gene. A control plasmid in which the same piece of phage λ DNA was inserted upstream of the promoter in pPOMCAT-84 was also generated (panel 1). The 97-bp *Bss*HII fragment was also inserted 1.09-kb downstream of the CAP site in one and two copies in pPOMCAT-84 (panel 4). The data presented in the 8Br-cAMP+IMX column refers to an 8 h treatment with 1.0 mM 8Br-cAMP+0.5 mM IMX and data in the TPA+IMX column refers to an 8 h treatment with 200 nM TPA+0.5 mM IMX.

Methods. Regulators were added to two plates of each group as described in the legend to Fig. 1. Data for *a* and *b* are normalized CAT activities for each plasmid relative to the activity of the first panel in the absence of regulators which was arbitrarily assigned the value of 1.0. The plasmid pPOMCAT-84 was constructed by replacement of the -193 to +100 region of pENKAT-12 with the *Eco*RI to *Pst*I region of the multiple cloning site from pUC18 and a *Pst*I to *Hinc*II fragment (-84 to +174) of the mouse *pomc* gene³⁵. The plasmid pENK-POM-1 (*a*, panel 5) was constructed by inserting a 109-bp *Eco*RI to *Pst*I fragment from positions -193 to -80 of the *henk* gene into the *Eco*RI to *Pst*I site of pPOMCAT-84. The plasmid pENKAPOMCAT (*b*, panel 2 and 3) was isolated by screening insertions of *Pst*I-cut phage λ DNA cloned into the unique *Pst*I site in pENK-POM-1. The remaining constructions were generated by the purification of the phage λ DNA *Pst*I insert and reinsertion into the *Pst*I site of either pENK-POM-1 or pPOMCAT-84. The *henk* 97-bp *Bss*HII fragment was inserted into a *Mlu*I site in pPOMCAT-84 1.09 kb downstream of the putative CAP site using the cohesive ends generated by *Bss*HII and *Mlu*I cleavages. The plasmid pENKTKCAT (*a*, panel 7) was generated by excising the *henk* *Bss*HII fragment from the modified pUC18 polylinker by cleavage with the restriction enzymes *Sac*I and *Xba*I and insertion upstream of the *Xba*I site of pUTKAT-3 (*a*, panel 6) at position -109.



the POMC promoter (Fig. 3b). Single or multiple copies of oligo A inserted in either orientation into the *Pst*I site of pPOMCAT-84 can confer inducibility by both cAMP and phorbol ester (Fig. 3b). Two copies of oligo A in either orientation function better than a single copy, a result similar to the requirement for multiple copies of the 12-bp metal regulatory element for efficient induction of the metallothionein-1 promoter by heavy metals²⁰. Oligo B, which alone is unresponsive to regulators, increases the activity of oligo A when placed either directly upstream or downstream of oligo A in pPOMCAT-84 (Fig. 3b).

A comparison of human¹³ and rat²¹ proenkephalin 5' flanking sequences indicates that a region with >95% homology is located between -108 and -28 bp 5' of the human proenkephalin gene. Sequences between -108 and -75 are perfectly conserved and contain three closely related repeats of a 12-bp sequence (boxed sequences shown in Fig. 3a). The region containing *henk* repeat units 2 and 3 (-96 to -78, see Fig. 3 legend) is similar to several sites within the 5' flanking DNA of the rat

phosphoenolpyruvate carboxykinase (*pepck*) gene²² (Fig. 3c), and to sequences found near the 5' end of the human prolactin²³, corticotropin releasing factor²⁴, proenkephalin B²⁵ and somatostatin²⁶ genes (Fig. 3c). The *pepck* and prolactin genes are known to be regulated by cAMP^{22,27}; regulation by cAMP for the others has not been reported. The *henk* 2-3 region matches at 11/12 bp with a sequence found between -300 and -289 of the human *c-fos* gene¹⁹ and with sequences in the polyoma enhancer²⁸.

Regulation by both cAMP and phorbol ester map to the same 29 bp region of *henk* 5' flanking DNA (oligo A, -100 to -71; Fig. 3a) suggesting that these two intracellular signalling pathways converge to regulate a common effector of transcription. Phorbol esters, thought to act exclusively by activation of protein kinase C (although other actions are possible), may act rapidly to increase intracellular levels of cAMP^{29,30}, activate the cAMP dependent protein kinase directly, or phosphorylate an effector of gene expression common to both pathways through protein kinase C.

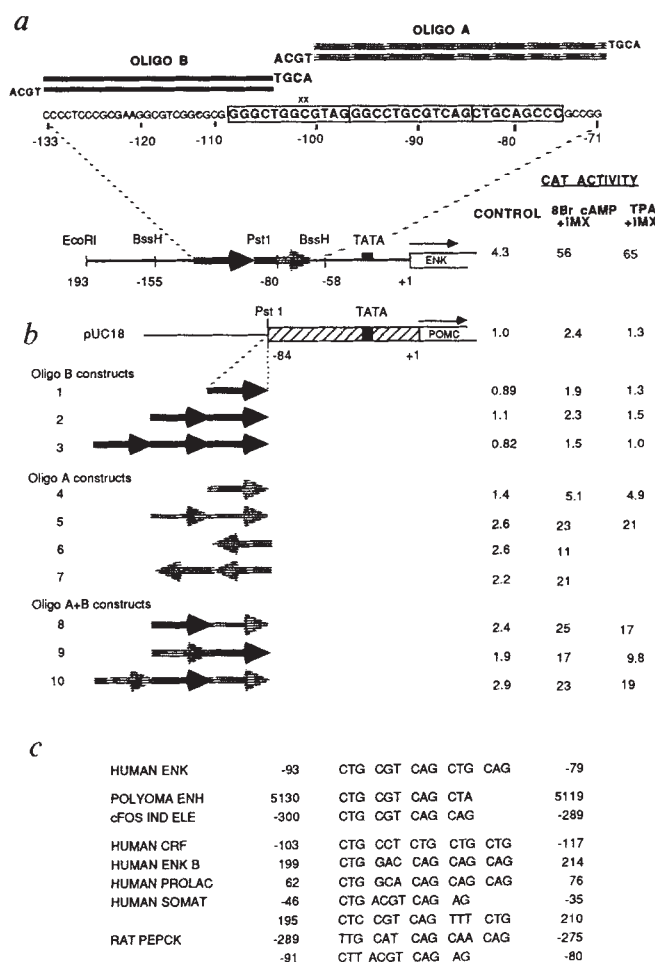


Fig. 3 Transfer of cyclic nucleotide inducibility to the mouse *pomc* promoter with oligonucleotides corresponding to two regions of *henk* 5'-flanking sequences. *a*, Regions of the *henk* 5'-flanking DNA to which double-stranded oligonucleotides were synthesized are indicated at the top. Oligo A corresponds to region -100 to -71 and oligo B to region -101 to -133. Oligo B contains a 2-nucleotide change at positions -101 and -102, changing GC to CA, and converting sequences between -105 and -100 into a *Pst*I site. This enables oligo A and oligo B to be ligated together at the new *Pst*I site with a 2-bp change at the junction. The boxed sequences correspond to the closely related 12-bp repeated sequences, *henk* 1 (-108 to -97), *henk* 2 (-96 to -85) and *henk* 3, present in the opposite orientation, (-76 to -87). *b*, Effects on cyclic nucleotide and phorbol ester regulation by the *henk* oligonucleotides inserted into the *Pst*I site (-84) of the mouse *pomc* promoter in pPOMCAT-84. The position of oligo A and oligo B in the *henk* 5' flanking DNA is indicated with oligo B as a solid arrow and oligo A as a hatched arrow. One, two and three copies of oligo B (lines 1, 2 and 3) were isolated as tandem repeats. Clones containing copies of oligo A (lines 4, 5, 6 and 7) or oligo A+oligo B (lines 8, 9 and 10) were isolated and CAT activities determined. Each plasmid was transfected as described in Fig. 1 legend. CAT activities were normalized to β -galactosidase activities and the relative CAT activity in the untreated plate transfected with pPOMCAT-84 (top) was arbitrarily assigned the value of 1.0. The data presented in the 8Br-cAMP or TPA columns refers to an 8-h treatment with 1.0 mM 8Br-cAMP+0.5 mM IMX or 200 nM TPA+0.5 mM IMX. *c* Homologies between the *henk* repeat sequence 2 and 3 region (-93 to -79) and several transcriptional enhancer elements and the 5' ends of several cAMP or potentially cAMP regulated genes. The sequence locations on the left refer to nucleotide positions relative to the mRNA cap sites in the references listed in the text.

Methods. Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer corresponding to the sequences in the *henk* promoter region described above. Additional nucleotides were added to each oligomer to generate *Pst*I sticky ends which were used in the cloning of the synthetic DNA fragments into the unique *Pst*I site at position -84 of pPOMCAT-84. The number and orientation of oligomers inserted into the POMCAT-84 was determined by restriction mapping and dideoxy-sequencing of supercoiled plasmid as described by Chen and Seeburg³⁶ using a M13/lac reverse 17mer as primer (New England Biolabs).

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Cumulative effect of intragenic amino-acid replacements on the thermostability of a protein

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The marginal net stability of a folded protein is thought to depend on a small difference between large, compensating individual forces. Therefore, the net free energy of stabilization of proteins is unexpectedly small (~ 10 kcal mol⁻¹)¹. The contribution of individual forces such as hydrogen bonds and salt bridges to the stabilization is evaluated as 1–3 kcal mol⁻¹ (refs 1, 2), and several additional forces are thought to be sufficient to account for the extra thermostability of thermophilic proteins^{2–5}. The native conformation of a protein is determined by the total number of interatomic interactions and hence by the amino-acid sequence⁶. If the few amino-acid residues that individually contribute to the stabilization could be implemented concurrently into the sequence, the multiple replacement would enhance the overall stability of the protein molecule. Here we report evidence to support this argument. Thermal inactivation kinetics and proteolytic resistance for mutants of a kanamycin nucleotidyltransferase reveal that a few intragenic amino-acid replacements stabilize the protein cumulatively. Our experiments not only demonstrate the feasibility of elevating the thermostability of a protein but also lead to better understanding of the forces that are responsible for protein stability.

Table 1 Specific activity and optimum temperature of KNTases

Enzyme	Specific activity* (%)	Optimum temperature† (°C)
WT	100	~52
K130	95	~57
Y80	85	~60
Y80/K130	80	~62

KNTase activity assayed in a reaction mixture containing [^3H]ATP and kanamycin⁸.

* Enzyme concentrations determined spectrophotometrically (molar absorption coefficient for WT KNTase, $\epsilon_{280\text{ nm}}^{0.1\%} = 1.55$). Specific activities of mutants at 37°C are presented as the per cent of WT enzyme (~190 pmol AMP-kanamycin per μg enzyme per 10 min at 37°C).

† Assay reactions were done at 17–72°C for 10 min and temperatures that maximize the formation of AMP-kanamycin are shown. Specific activities at each optimum temperature are nearly the same (~510 pmol per μg per 10 min) for all enzymes.

A kanamycin nucleotidyltransferase (KNTase) transfers a nucleotide residue to the hydroxyl groups of amino-glucose moieties of kanamycin and inactivates the antibiotic⁷. The enzyme is a monomeric protein consisting of 253 amino acid residues⁸. Recently, we have identified two sorts of single amino-acid replacement, Asp 80 $\rightarrow$ Tyr (Y80) and Thr 130 $\rightarrow$ Lys (K130), that individually enhance the thermostability of KNTase⁹. A temperature-sensitive (*ts*) mutant, Pro 252 $\rightarrow$ Leu (L252), has also been isolated¹⁰. The thermostable (Y80 and K130) and thermolabile (L252) mutants were used in addition to the wild-type (WT) enzyme as the starting materials.

We constructed, first, a double mutant (Y80/K130) by conversion of Thr 130 (ACG) to Lys (AAG) within Y80 enzyme using oligonucleotide-directed mutagenesis¹¹ and, second, one triple mutant (Y80/K130/L252) and two double mutants (Y80/L252 and K130/L252) having Leu 252 (CTA) in common (Fig. 1). In plasmid pUC12kan, the expression of KNTase is controlled by a *lac* promoter-operator and transformants in *Escherichia coli* JM101 (F'*lacI*^q)¹² overproduce the enzyme when isopropyl-thio- β -D-galactoside is added. All the mutant and WT KNTases were purified and their thermostabilities examined.

We studied the irreversible thermal inactivation kinetics for KNTase at several temperatures and plotted the first-order denaturation rate constant (k_d) against the reciprocal of the absolute temperature (T^{-1}) (Fig. 2). Taking, for example, the same value of k_d of 0.1 min^{-1} in each KNTase, the single replacement by Lys 130 (K130) or by Tyr 80 (Y80) increases the specified temperature for WT enzyme to an extent of 3.8 (53.5–57.3) or 7.4°C (53.5–60.9), respectively. This increase of temperature will hereafter be called the enhancement of thermostability. The simultaneous introduction of residues Lys 130 and Tyr 80 (Y80/K130) enhances the thermostability of WT enzyme by 10.8°C (53.5–64.3). Thus, the combination of both residues seems to function cumulatively in terms of thermostability. This apparent cumulative effect of Tyr 80 and Lys 130 on the WT enzyme stability is also observed when the *ts* mutant L252 is used as a basis of the mutant constructions (see L252, K130/L252, Y80/L252 and Y80/K130/L252). Here, note that a specific combination of only three residues in the 253-amino-acid enzyme altered the thermostability by as much as 18°C in this example (see L252 and Y80/K130).

Resistance to proteolysis is another measure of the stability of a protein, because denatured proteins become more sensitive to proteolysis than the native ones¹³. We compared the resistance to digestion by thermolysin among WT and mutant KNTases (Fig. 3). It is evident from Fig. 3 that a single, thick band corresponding to each intact KNTase diminishes at higher temperatures depending on the thermostability enhanced: the double mutant (Y80/K130) is more resistant to the digestion than the single mutant (Y80 or K130). The band for the native WT enzyme decreases at temperatures between 53 and 56°C.

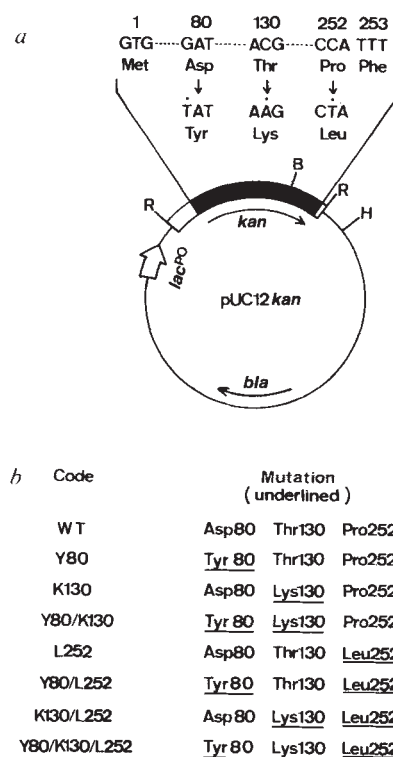


Fig. 1 **a**, Schematic representation of the recombinant plasmids used for expression. An *EcoRI* DNA fragment (930 base pairs, bp)⁹ including a KNTase gene (*kan* gene, 759 bp)⁸ (closed bar) and its flanking regions (open bar) was cloned into the polylinker site of pUC12 (ref. 12). The cloned *kan* gene was expressed under the transcriptional control of the *lac* promoter-operator (*lacPO*) on the vector. Arrows inside pUC12kan denote the direction of the *kan* and β -lactamase (*bla*) genes. R, B and H are restriction sites of *EcoRI*, *BglIII*, and *HindIII*, respectively. Taking the first residue (Met) of KNTase as 1, only the relevant residues are shown. The base of each mutation is indicated by an asterisk. **b**, Wild-type and mutant (Y80, K130, Y80/K130, L252, Y80/L252, K130/L252 and Y80/K130/L252) KNTases. Mutations in each KNTase are underlined.

Methods. The single-stranded template (+) DNA of M13mp10amkan(Y80) (ref. 9), which contains amber mutations in phage genes I and II, was prepared and mixed with *EcoRI*-digested replicative-form (RF) DNA of M13mp10w (ref. 10) that lacks the amber mutations. After heat denaturation and hybridization, the gapped-circular heteroduplex was constructed as described previously¹⁰. An oligonucleotide 5'GTGGAACCTTTGGGC3' was synthesized⁹ for the Thr 130 to Lys mutation. The 5' phosphorylated mutagenic primer was annealed to the heteroduplex, extended with DNA polymerase I Klenow fragment and ligated¹¹. *E. coli* JM105 (suppressor-free strain)¹² was transformed by the repaired DNA, and phage progenies derived from (–)-strand, on which the mutagenic primer was incorporated, were segregated on the lawn of strain JM105¹⁰. Following preparation of single-stranded DNAs, the mutants (Y80/K130) were screened by dideoxy sequencing with only one dideoxynucleotide in the sequencing reaction¹⁸. The mutant was then verified by sequencing the entire *kan* gene⁹. The RF DNA of M13mp10wkan(Y80/K130) was prepared and the *kan*(Y80/K130) gene transferred into pUC12. The recombinant plasmid, pUC12kan(Y80/K130), was digested with *BglIII* and *HindIII*, and then a large fragment (~3,400 bp) containing the vector sequence and the major portion of the *kan* gene including the codons Tyr 80 and Lys 130 was prepared by 1% low-melting temperature agarose gel electrophoresis¹⁹. Separately, a short *BglIII*/*HindIII* fragment (~200 bp) containing the codon Leu 252 in the *kan* gene was prepared from pUC12kan(L252) by 4% polyacrylamide gel electrophoresis¹⁹. Following the ligation of both fragments, the recombinant plasmid pUC12kan(Y80/K130/L252) was isolated. Similarly, pUC12kan(Y80/L252) and pUC12kan(K130/L252) were constructed using pUC12kan(L252) and either pUC12kan(Y80) or pUC12kan(K130) (ref. 9).

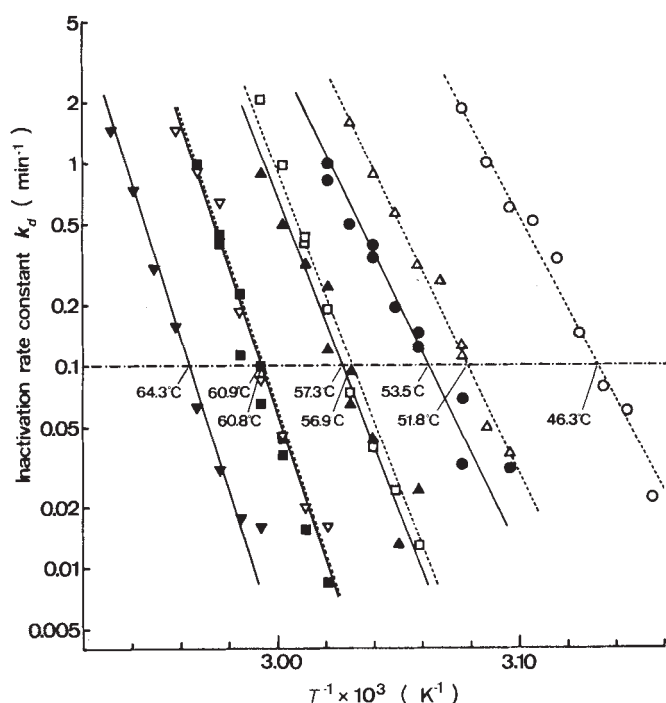


Fig. 2 Arrhenius plots of thermal inactivation rate constants of KNTases. ●, WT; ▲, K130; ■, Y80; ▼, Y80/K130; ○, L252; △, K130/L252; □, Y80/L252; ▽, Y80/K130/L252.

Methods. All KNTases were purified from *E. coli* JM101 (*F' lacI^q*) (ref. 12) carrying the appropriate pUC12kan. Cells were grown in YT broth (ref. 20) with kanamycin (30 µg ml⁻¹) at 37 °C. When cells were grown to ~0.6 (*A*₆₆₀), isopropyl-thio-β-D-galactoside (0.2 mg ml⁻¹) was added and the cultivation continued for a further 3 h. KNTase was purified to ~95% homogeneity by gel filtration (TSKgel G3000SW, Toyo Soda) by ion-exchange chromatography (TSKgel DEAE-5PW) as described¹⁰, and by hydrophobic chromatography (TSKgel Phenyl-5PW) with a linear gradient from 500 to 0 mM ammonium sulphate in buffer N (ref. 8) without glycerol. The purified enzymes were dialysed against buffer N. Small test tubes containing 100 µl enzyme (~100 µg ml⁻¹) were incubated at various temperatures. At intervals one of the test tubes was taken out and cooled quickly in iced water. The remaining KNTase activity was assayed as described⁸. The first-order denaturation rate constant (*k_d*) was assessed from the slope of a semi-logarithmic plot.

This suggests that the native conformation of WT enzyme is denatured at these temperatures, and hence the enzyme becomes more digestible. This interpretation is supported by an assessment that ~35% of the native WT enzyme remains when exposed to 53 °C for 15 min, whereas only ~0.5% remains at 56 °C when exposed for the same period (assessed from Fig. 2, taking *k_d* values of 0.07 and 0.35 min⁻¹ at 53 and 56 °C, respectively). Note that the thermostabilities for WT and mutant KNTases in Fig. 2 are all consistent with the thermolysin assay of these KNTases in Fig. 3.

Thermostable enzymes from thermophiles are generally less active than those of mesophiles at optimum temperatures of the latter¹⁴. Thus, we examined the catalytic properties of WT and mutant KNTases. We find the specific KNTase activities of the mutants to deteriorate slightly as the thermostability increases, but the optimum temperature of KNTase is shifted upwards with the increase of thermostability (Table 1). The activation energy of the mutant KNTases for the catalysis is nearly the same as that of WT enzyme (~11 kcal mol⁻¹). These observations indicate that the mutant KNTases with single or double amino-acid replacement(s) acquire the extra thermostability without significant loss of catalytic efficiency. This finding is supported by the report that a *ts* mutation of T4 phage lysozyme does not disturb the gross three-dimensional structure of the protein¹⁵.

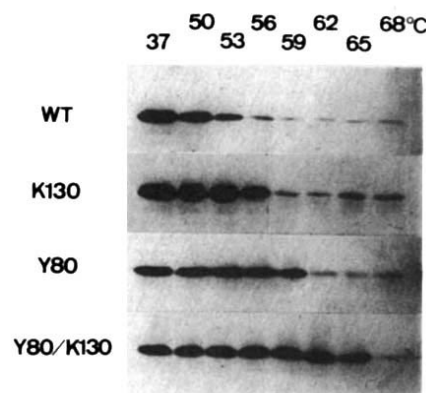


Fig. 3 SDS-polyacrylamide gel electrophoretic analysis of proteolytic resistance of KNTase. Each panel shows digestion with thermolysin at eight different temperatures.

Methods. Purified KNTase was digested with thermolysin at enzyme-substrate ratio of 1:50 (w/w) in a buffer containing 15 mM Tris-HCl (pH 7.8), 10 mM MgCl₂, 5% glycerol, 0.1 mM dithiothreitol, 25 mM NaCl and 25 mM CaCl₂. Reactions were performed for 15 min at the temperatures shown and were terminated by adding EDTA to 10 mM. Digestion products were electrophoresed on 12.5% SDS-polyacrylamide gels²¹.

As for forces of the stabilization in KNTase, extra hydrophobic interactions and hydrogen bonding caused by the Tyr 80 residue⁹ as well as extra electrostatic interaction resulting from the Lys 130 residue^{8,9} might contribute to the cumulative thermostability of the Y80/K130 enzyme. But this cumulative effect would not be limited to elevation of the thermostability. The *ts* mutation (L252) triggers the reduction of thermostability not only in WT enzyme but also in all the thermostable mutants (Fig. 2). These findings suggest that the individual residues of Tyr 80, Lys 130 as well as Leu 252, might behave almost independently to stabilize and/or destabilize KNTase in thermal denaturation.

One object of protein engineering is to improve enzyme properties such as thermostability, temperature optimum and protease resistance¹⁶. Our studies could be applied to other enzymes as long as the amino-acid residues responsible for these properties are identifiable. In this context, the development of a screening system^{9,17} to allow the identification of key residues is important. The present study also provides a direct measure of the stability analysis of a protein.

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NEW JOURNALS

Altered points of reference

Mary Holmes on what has — and has not — changed in the library since J.D. Bernal proposed sweeping revision of the means of disseminating scientific information.

NINETEEN eighty was a depressing year. It started with the invasion of Afghanistan, continued with unrest in Iran and ended with the subscription prices of journals, which looked as if they had reached a plateau after their dramatic climb through the 1970s, again leaping upward. To compound the feeling of distraction among librarians, politicians decided it was time to reduce library budgets.

In 1972, reviewing prices of physics journals from 1959 to 1969, M.J. Matarazzo wrote "Increases in subscription rates for scientific journals have been so marked as to appear astronomical". During that time prices had risen 202 per cent, though the number of pages had also increased by 147 per cent. One journal, *Nuclear Physics*, which had produced four volumes per year in 1959, had reached the dizzy heights of an annual output of 23 volumes by 1969. From the mid-1960s molecular biology and biochemistry rivalled chemistry and physics as the most expensive journals and outstripped them with new titles: 45 per cent of the titles in 1979 did not start publication until after 1967. From 1971 to 1986 the prices of scientific and technical journals have continued to rise on average 16 per cent each year. The highest rise was 27.5 per cent from 1976 to 1977, and the lowest 6 per cent from 1979 to 1980.

The response of most libraries to slashed budgets was to reduce their staff and to cancel journal subscriptions, leading to publishers further increasing subscription prices. Budget cuts, introduced in other countries earlier, hit Germany in 1981 and 1982. University libraries suffered total budget reductions of DM 18.7 million in 1981 and in 1982 13 of these libraries had between them to cancel 7,265 periodicals. Cuts have continued, with no country being immune to them: this year the Library of Congress has had its budget cut twice.

Then, of course, there is the growth in titles. The first scientific journals were published in 1665. *Le Journal des Savants* came out in Paris in January, followed in March by the *Philosophical Transactions of the Royal Society* "giving some account of the present undertakings, studies and labours of the ingenious in many considerable parts of the world". The first number consisted of 16 pages, and reported on astronomy, "optick glasses in Rome; lead-ore in Germany; the relation [sic] of a very odd monstrous calf", and the books of M. Fermat "lately dead". By the beginning of the eighteenth century there were about a

100 journals, by 1900 the number was 10,000. In 1975 the British Library's "Current Serials Received" listed 45,000 journals. In the 1986 edition 70,000 current journals are listed with the announcement that there are another 2,500 on order and that in addition 164,000 non-current serials are available. At this growth rate there should be 20,000,000 by the year 2050!

With more—and more expensive—journals, and less to spend on them, how are beleaguered libraries to cope? Until



Room for reading — the British Museum Library, c. 1902.

the end of the 1940s the means of storing and retrieving information had not changed dramatically from those used in the library at Alexandria. In 1948 the Royal Society of London convened a conference to examine possible improvements to the collection and distribution of scientific literature. J.D. Bernal spoke at the conference and his comments are still relevant:

What is wrong with the present system is that the growing abundance of primary scientific publication and the confusion in which it is set out acts as a continuous brake . . . to the progress of science. We are not so much maintaining that scientific information is lost, but that the scientific worker wastes time and effort finding what information there is.

Bernal proposed a revolutionary plan for setting up national distributing authorities to which a short version of all scientific papers should be submitted. After going

to referees, "for not longer than a week", these papers would be published as pre-prints and distributed to individual subscribers interested in the field and, bound together in journal form, to libraries. The full form of the paper would be reproduced on microfilm for storage in libraries and distribution according to demand.

Twelve years later, remarking that it had been quicker to pass on scientific information at the time of Galileo, Bernal made an even more radical proposal:

. . . the abolition of scientific papers themselves . . . the facts and ideas which go to make up the paper, could be communicated directly. . . . This kind of communication will certainly come with the development of the computing machine.

Since hearing Bernal speak with enthusiasm and visionary zeal in the late 1950s on the advantages of a microform library, I have often thought wistfully of microfilm while performing prodigious feats of weightlifting in transporting journals. Some satisfactory microform libraries have been established, particularly in the United States, but it has never become popular in Europe. The lack of standardization (16mm and 35mm film and fiche in various sizes and shapes) has been discouraging. One has also to surround oneself with a workshop of hardware devices for reading and copying and the end product is often a rather damp, pale grey, pungent copy whose plates are indecipherable.

In many of the changes to the form and content of journals, one does see the realization of Bernal's ideas. In 1977 the Chemical Society of London launched the *Journal of Chemical Research* with the Chemical Societies of France and Germany as fellow founders. This journal comes in two parts, Part S which is synopses of papers and Part M the full text in microfiche or miniprint. The "Letters" journals for rapid publication of short reports or preliminary communications fulfil the two requirements of speed and brevity.

More than anything else, however, it is the development of the photocopier, in the 1940s still a pipedream, that has made individual articles widely available, whether from the library down the road or from the large national libraries. It is difficult to remember how we survived before the age of the photocopier. The heat and smell of ozone and the perpetual whirring from the photocopy room are an expected part of most libraries. Publishers complain about photocopying, but to their advantage a large number of photocopy requests for a specific journal can provide justification for subscribing. It would also be difficult to woo scientists to publish in new journals if they felt their papers would only reach 500 or so subscribing libraries — the number that is said

to be necessary to make a journal viable.

As Bernal predicted, the "computing machine" does indeed provide direct communication. With ever-improving networks and computer mail, information and files flow backwards and forwards between scientists throughout the world. We sit at our terminals and read articles from *Journal of the American Chemical Society* or *Macromolecules*. Nevertheless there are still many scientists who, however comfortable the chair, have no wish to absorb information from a visual display unit or a tatty print out. Where the computer has become indispensable is for the retrieval of scientific literature. An explosion that has been even more dramatic than that of printed literature is that of online databases. In 1970 there were only a handful — today there are between 3,000 and 3,500, and approximately 450 "hosts" that operate them. This new technology is expanding the capabilities of libraries which, having had to cut their own acquisitions, have now much better access to a wide range of journals held by other libraries. With the help of online bibliographic retrieval scientists are made aware of articles published in less well known journals. Libraries spend more every year to buy a decreasing percentage of the scientific output, but with the help of computer technology scientists can be kept informed of new publications, holding libraries can be located and copies ordered online. The electronic text delivery systems that are being developed will reduce document delivery times to hours and minutes rather than days and weeks.

It will be interesting to see how the library looks in the year 2000. So far, for all its power, electronic communication has not supplanted conventional journals and several features of life in the library could well remain. It would be sad if we no longer received in paper form those journals which sit in neat matching rows starting with volume 1. But will there be the same daily irritations associated with printed material? For instance finding that the journals which arrived this morning have, within hours, been transformed into loose-leaf format ("gluing" is cheaper than "sewing"!); that the issue containing hot news on the latest oncogene has disappeared; that one journal, that shall be nameless, no longer issues the indexes separately but glues them firmly into the next volume (this index must be photocopied, for what librarian would dream of tearing a journal to pieces to extract the index?). I suspect so. For while prophets continue to predict the apocalypse, we are just writing off, with librarian-like gullibility, for sample copies of all those "indispensable" journals due to appear this autumn. □

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New journals June 1984 to May 1985

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:

- (i) the first number appeared, or the journal was re-titled, between June 1984 and May 1985 (although journals not covered in last year's review issue were also considered);*
- (ii) the journal is published at least three times a year;
- (iii) the main language used is English;
- (iv) where possible at least four issues should be made available for review, including the first and the most recent numbers.

Last year's journals review supplement covered publications appearing between June 1983 and May 1984 and the second cut-off date, May 1985, allows for enough issues of a journal to have been published for a reasonable sample to be available for judgement (most are quarterlies). A

*See *Nature* 317, 293–308 (1985). For previous journals review supplements see *Nature* 311, 309–330 (1984); 305, 477–497 (1983); 299, 491–514 (1982); and 293, 341–369 (1981).

spread of four different issues is taken as the usual minimum on which reviewers' comments can be based.

Several journals thought to satisfy the above criteria were not submitted for review, or arrived too late for inclusion. It proved difficult to find reviewers for other, doubtless worthy journals, while some titles were considered to be of marginal interest to *Nature's* audience.

The brief given to reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample, and apply to mid-1986 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1987. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned. □

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Putting science on the map

Leonard Krishtalka

National Geographic Research. Editor Harm J. de Blij. *National Geographic Society.* 4/yr. US \$40; Canada \$61.85; elsewhere \$45 (surface), \$60 (air).

GEOGRAPHY is the science of place. Its vision is grand, its view panoramic. It sweeps the surface of the Earth, charting the physical, organic and cultural terrains, their areal differentiation, and their ecological dynamics with man. Its foremost tool is the map.

Ever since its founding in January 1888, the National Geographic Society has remained faithful to the romance of geography. From the lead article in the inaugural *National Geographic Magazine* in October 1888 ("Geographic Methods in Geologic Investigation") to the last one in the July 1986 issue ("Model Airplanes: and then to Fly"), its sense of geography has remained classical: the physical contours and features of the continents and oceans; life on Earth, past and present; human cultures, traits and economies; the evolution of man; the geography of space beyond Earth; and, of course, smashing colour photography and so many fine maps.

In keeping with its charter ("for the increase and diffusion of geographic knowledge"), the society began funding geographic exploration and research just after its inception. As of this year, it has supported over 3,000 such projects, notably Peary's final assault on the North Pole, Byrd's expeditions to the South Pole, the archaeological unearthings of Machu Pichu (Peru) and Chaco Canyon (USA), two generations of Leakeys in the badlands of the East African Rift (Kenya and Tanzania) prospecting for the origin of man, Cousteau charting undersea life, Goodall charting chimpanzee life (Tanzania), the American Mount Everest expedition, and the flights of the stratosphere balloons, *Explorer I* and *Explorer II*.

These are the seeds of *National Geographic Research*, a new scientific journal devoted primarily (but not exclusively) to publishing the results of the studies that the Society funds. Ironically, the first issue (Winter 1985) fulfilled a 76 year-old resolution by the Board of the National Geographic Society to "undertake the publication of a technical geographic journal to be separate from the *National Geographic Magazine*". Until 1985, grant recipients summarized their findings in the Society's annual *Research Reports*, a tome that is the very antithesis of geography: a ledger of stiff, dry tracts devoid of maps or photographs.

Not so *National Geographic Research*, the handsomest, most elegant professional scientific journal known to me. Its

origin from the musty *Research Reports* is a remarkable speciation, and it finally puts the lie to Dawson's axiom that if it's handsome, it's art; if it's science, it's dull. What a magnificent revelation to see hard science in colour and not on public television. Natural science is usually a comic duality. In the field it studies a universe of magisterial colour; in print it becomes a stark black and white. But not here. *National Geographic Research* is a dazzling display of natural history in polychrome. The drawings, charts, maps and photographs, many of them prepared by the Society's art staff, are vivid with colour and are worth the proverbial thousand words. They impart the immediacy of an eye witness account, the excitement of science in the flesh. As a result, the few black-and-white illustrations in each issue, although superior, are a comedown. Of course, fine artwork demands, and receives, the finest paper.

If the style is a knockout, what about the substance? The knee-jerk tendency among too many academics is to succumb to a kind of elitism that afflicted the "serious" art world long ago, namely, the critical platitude that the attractive or popular cannot be serious. Again, not here. The "research" in *National Geographic Research* is as solid and scholarly as that in any first-rate scientific journal, if not more so; each paper must pass the muster of at least two outside referees to be considered for publication. The Board of Editors, which reads like a Who's Who of natural history, and the editor Harm J. de Blij, have reminded us of the matching valence of erudition and beauty in science and its communication.

The writing in *National Geographic Research*, for the most part, is unusually lucid, perhaps because the authors are aware of the journal's broad scope and audience and are willing to make the effort to make their geology, for example, understandable to an ethologist without sacrificing the subtle technical shadings of the science. The cross-disciplinary breadth of *National Geographic Research* is true to its geographic roots and a refreshing antidote to libraries stacked with specialized journals. The seven issues to date have included articles on vertebrate palaeontology, archaeology, ethology, urban anthropology, ethology, ecology, palynology, zoology, geology, historical geography, primatology, entomology, biogeography and botany. The connecting thread is the science of place.

Each issue of *National Geographic Research* has four sections: "Articles", for longer reports; "Forum", for shorter papers; "Noted Elsewhere", for capsule summaries of Society-funded research published in other journals; and "Letters to the Editor", where authors can also rebut critics. In a nice touch that is loyal to the geographic tradition, the title page of each article features a colour globe with the appropriate geographic area demarcated by a yellow rectangle, the insignia of the Society.

National Geographic Research, like its less technical but more famous cousin, *National Geographic Magazine*, is entirely supported by subscriptions. The latter, a veteran of 98 years, boasts an audience of over three million; the former, a rookie, reached 4,000 subscriptions at last tally (the Winter 1985 issue). That 4,000 number should grow, if only because grand science in great colour should seduce the libraries, the curious and all practitioners of natural history and classical geography. □

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Public talk about human affairs

Paula Brown Glick

Anthropology Today. Editor Jonathan Benthall. *Royal Anthropological Institute*, 56 Queen Anne Street, London W1M 9LA, UK. 6/yr. UK £10 (institutional), £7 (individual); North America \$14 (institutional), \$12 (individual).

THE Royal Anthropological Institute has revised its journals format more than once since 1965. *Anthropology Today* is an enlarged and illustrated successor to *Royal Anthropological Institute News (RAIN)*, while the quarterly *Man*, is devoted to longer articles, reviews and related correspondence. An amalgam of news, announcements, reviews, short research articles and commentary, *Anthropology Today* is to be distinguished by "its orientation towards the world outside: that is, towards public and topical issues and towards other professions". The journal seeks to inform about the work of anthropologists, to cover all tendencies and movements, to debate issues, and to carry information of both national and international concern.

What might this new-found public for anthropology find of interest? Minorities, refugees, ethnic conflict, victimization of tribal peoples are subjects on which anthropologists often have field experience as well as concern: Survival Inter-



national's objection to the Museum of Mankind's exhibition on Amazonian Indians; the plight of the Miskitu Indians of Nicaragua; Christianity in China; child labour; and Hindu-Moslem confrontation are among the topics discussed in the issues that have appeared to date. Ethnographic films, a meeting-ground of anthropologists and the public, are a favourite subject for discussion and review. Study of other professions, and their interaction with anthropology, is exemplified in articles on development economics, clinical practice, psychiatry and body alteration.

It seems likely that *Anthropology Today* is, and will be, seriously and selectively read by anthropologists for their specialty: book reviews, reports of conferences, research and assessments of recent work in, for example, ceramics, feminist anthropology, human biology or religion. Obituaries and memoirs of anthropologists have been a rich field, especially in the centennial year of Malinowski's birth. Critical opinions stimulate response, correction and debate. The news of people, exhibitions, conferences, films, books, positions available fill one-quarter or more of the space, and there is also a UK insert (which I have not seen) for news of interest to readers in Britain.

Such a magazine is as interesting, selective or broad as its editors and contributors make it. Format and design are attractive, with scope for photographs, diagrams and maps. The language is non-technical, but not always lively. In its first year or two, even as a successor to a more limited periodical, it has the difficult task of finding a role valued by the profession and the public. However, there is no doubt that *Anthropology Today* does provide an informal, international forum for the varied interests and viewpoints of professional anthropologists and other commentators, unmatched in any other English-language publication. If it seems to be a bit of everything, and something for everyone, this may be a reflection of anthropology today. □

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Simply the place to be seen?

Warren R. Jones

Advances in Contraception. Editor-in-chief G.I. Zatuchni. MTP Press. 4/yr. Dfl. 210, £52.50, \$86.

RESEARCH and development in contraception, by and large, is in the doldrums. The majority of so-called "advances" in contraceptive technology are modifications of existing methods or attempts to improve their safety, acceptability and distribution. The contents of *Advances in Contraception* confirm this rather depressing outlook.

One of the four issues I received was wastefully devoted to abstracts of an annual meeting of the Society for the Advancement of Contraception, for whom the journal acts as official organ. Of the 27 articles in the other three issues, 23 were devoted to clinical studies or trials of contraceptive agents (12 on the IUD) and four were concerned with basic research in reproduction. Unless this emphasis changes, the journal will run the risk of limiting its readership to the professional purveyors of contraceptive trials, many of

which are underwritten by pharmaceutical companies whose commercial capabilities are now, for the most part, restricted to keeping their products visible in the scientific and pseudoscientific literature. *Advances in Contraception* shows no evidence to date that it will attract contributions from researchers at the few sharp edges of contraceptive development.

On the plus side, the journal is well produced, with clear tables and half-tone figures. The abstracts are helpfully repeated in French and Spanish at the end of each paper; however the references are perversely numbered in sequence of appearance in the text. Reviews and letters are solicited but thus far only the former have appeared.

I suspect that *Advances in Contraception* has a preordained destiny that will allow it to survive the marketplace pressures on new scientific journals. The still vast international network of contraception-watchers will subscribe to, nurture and sustain it. For my part, I will economize in time and money and seek out one or two comprehensive reviews of clinical contraception every two or three years. □

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Help from outside

C.R. Austin

Journal of In Vitro Fertilization and Embryo Transfer. Editors-in-chief J. Friberg and N. Gleicher. Plenum. 6/yr. US \$130 (institutional), \$49.50 (individual); elsewhere \$146 (institutional), \$57.50 (individual).

WITH the phenomenal upsurge of interest and involvement in the production of "test-tube babies", the appearance of a highly specialized publication like the *Journal of In Vitro Fertilization and Embryo Transfer* was both justified and welcome. Beginning with four issues a year in 1984, it moved to six issues this year. Previously, most communications on the topic would have been offered to the *Journal of Reproduction and Fertility*, to *Fertility and Sterility* or to *Biology of Reproduction*, but there are clear advantages in gathering the material under one title.

The journal accepts original reports of basic or clinical investigations on human or animal subjects, as well as reviews and short communications. In general, a commendable standard has been maintained in the published papers, and one frequently sees names of workers known for their contributions in this field. A "News and

Views" section contains brief statements requiring rapid publication, as well as a most useful feature — a regular list of IVF-ET programmes recently "registered" with the central editorial office. Judging by the contents of the April 1986 issue, the journal also publishes (in the rather unsatisfactory camera-ready format) abstracts of papers read at appropriate conferences: thus in April, there were 82 abstracts from the Congress of Future Aspects in Human In Vitro Fertilization, held in Vienna in April 1986. Another important service is the organization of symposia specifically for the journal. The February 1986 issue contained papers making up one such symposium on "Embryo Freezing"; and a very interesting and useful compilation it was, too, dealing with general principles, and work on mouse, baboon and human embryos. Curiously, the symposium organizer was identified as "guest editor", though an associate editor of the journal.

Delays in the publication of regular papers and even of short communications have been rather long, roughly 3–7 months from "acceptance". Dates of "submission" have been included only since April 1986, and from a perusal of these it emerges that the total delay ranges from 8 to 15 months. It is surely important for the journal's future that this be improved. Tables, line drawings and half-tone illustrations are all clearly presented;

the journal makes no page charges. The general layout and the individual papers are also good, but there are some improvements the editors could make. For one, it would be more helpful to readers if the contents were listed on the front of the cover, rather than the array of editorial personnel, which also appears on the inside of the cover. Similarly, it would be more useful if the reference at the top of the first page of each paper included first and last page numbers instead of the part number. Finally, I would prefer to see full titles of journals instead of abbreviated forms in the references at the end of each paper.

A more recent addition to the journal supply is *Human Reproduction*, published by IRL Press, which first appeared in January 1986. It has the particular interest of being the official organ of the newly formed European Society of Human Rep-

IVF JOURNAL OF IN VITRO FERTILIZATION AND EMBRYO TRANSFER

roduction and Embryology, and, published only in English, will make the European contribution to this field of inquiry more accessible for the less linguistically accomplished. Among the journal's other attractions are that abstracts of papers presented at the first annual meeting of the society are published as a supplement and printed in standard letterpress, and that, so far, publication has been remarkably rapid — all 35 papers in the first three issues were published between two and five months from the time of their receipt. □

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Rhythmic research

Steven H. Strogatz

Chronobiology International. Editors Alain Reinberg and Michael Smolensky. Pergamon. 4/yr. UK £60 (institutional), £31 (individual); North America \$66.37 (institutional), \$51 (individual).

WE ALL have clocks inside of us. These internal oscillators control the daily cycles of body temperature, sleep and wakefulness, hormone titre, metabolism and so on. Chronobiology is the study of such biological rhythms and the endogenous clocks which generate them. Although physiological oscillations with a circadian or near 24-hour period have been identified in practically all eukaryotes studied, including some unicellular organisms, the molecular mechanisms behind these rhythms remain deeply mysterious.

In the past, research articles about chronobiology have been published in journals of ecology, genetics, physiology, statistics, cell biology, medicine, pharmacology, neurobiology, biomathematics and endocrinology. In other words, those of us concerned with biological rhythms have perforce become familiar with most of the journals in the biology library. *Chronobiology International* seeks to give rest to the weary.

Each issue contains about eight research articles, mostly concise reports of experimental work, often in pharmacology or endocrinology. Experimental subjects range from humans and rats to flies and *Euglena*. In the journal's first two years, few theoretical articles have appeared, and only one review article and one letter to the editor. Occasionally there are book reviews, meeting reports or announcements. European contributors

outnumber North Americans by about two to one, although the editorial board is more balanced.

The articles vary in quality. Many are well-written, first-rate pieces of work. On the other hand, in a journal devoted to rhythmic phenomena, it is disappointing that so many of the statistical analyses involve little more than the calculation of the best-fitting cosine. In fairness to this journal, however, an unsophisticated approach to time-series analysis pervades the chronobiology literature as a whole.

There are some refreshing editorial policies. During the review process, the authors remain anonymous to the referees. No author may be listed on more



CHRONOBIOLOGY INTERNATIONAL

Edited by
Alain Reinberg & Michael Smolensky

than three papers in any one annual volume, to ensure that others have a fair opportunity to publish. Articles are published about three or four months after acceptance.

The format is good, and figures are reproduced clearly, with ample space. Unfortunately some papers contain figures with clotted or miniscule lettering — probably the author's fault, but worthy of editorial attention.

With some tightening of standards on the part of the referees, *Chronobiology International* could provide a respectable home for research articles concerned with biological rhythms. □

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Programs to solve problems

S.W.D. Steel

Journal of Automated Reasoning. Editor-in-chief L. Wos. Reidel. 4/yr. Dfl. 233, \$97 (institutional); Dfl. 100, \$39 (individual).

AUTOMATED reasoning, states the Information for Authors of this new journal, is "a field whose objective is the design and implementation of a computer program that serves as an assistant in solving problems and in answering questions that require reasoning". That seems a natural and important constituency, and the papers so far published come from it.

The field is, of course, a continuum of subjects from the theoretical to the practical, and the journal reflects this. There are highly formal contributions on such topics as unification and intelligent backtracking that are important in actual programs throughout automated reasoning, and also papers about programs intended to solve particular problems (in planning, scheduling and expert systems, for example) which pay particular attention to theory to show why the inferences made are the right ones. The extrema, pure mathematical logic and pure program de-

tails, are rightly missing, though from the evidence of later issues there is the danger that formal papers might come to overwhelm contributions dealing with systems and algorithms. That would be a pity. As far as I can judge, the material published to date has been both substantial and interesting.

Authors are allowed sufficient space to explain the interest of their work and relate it to that of others (though not all of them do so). They can thus both present new research to specialists and introduce established work to those in other areas of artificial intelligence and computer science; with the paucity of intermediate- and higher-level textbooks on the subject, this is a valuable feature. For the same reason, the "Problem Corner" for passing on automated reasoning folklore is a good idea.

Given the level of interest in the field, artificial intelligence has not spawned as many journals as one might have expected

and some that do exist have consisted of little more than advertising. Instead there has been heavy reliance on the proceedings of a few highly regarded conferences and on the flagship journal *Artificial Intelligence*. These, as well as books about particular systems, could provide some competition for the new journal, but it is subsumed by none of them. It is clear there is enough good work to fill another journal; conference articles are too short to be expository; and books are for those who already know what they need.

My single reservation is about the poor proof-reading. Only errors in the prose actually strike one, but they give rise to doubts about the accuracy of the symbolic passages. That apart, *Journal of Automated Reasoning* is a much-needed publication that has made an auspicious start. □

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Nervous behaviour

Peter Bryant

Developmental Neuropsychology. Editor Francis J. Pirozzolo. Lawrence Erlbaum. 4/yr. North America \$65 (institutional), \$25 (individual); elsewhere \$74 (institutional), \$34 (individual).

THE main concern of developmental psychology is change. The behaviour of young children, and of old people too, changes rapidly and often quite dramatically over time, and these changes must be related in some way to changes in the central nervous system. In fact many of the neurological developments that take place with age are very well documented, but their exact relationship to the development of behaviour remains a mystery.

The subject is a difficult one for a number of reasons, but one barrier has been the lack of a suitable forum for discussion. Papers on the subject tend to end up in journals of developmental psychology, which are not read much by neuropsychologists, or in specialist journals of neurology or neuropsychology. One journal, *Developmental Medicine and Child Neurology*, has been remarkably successful and does cross this boundary, but it has concentrated on clinical topics. This new journal, *Developmental Neuropsychology*, spreads its net more widely, covering normal as well as abnormal development.

Much of the information that we do have on the possible relations between neurological and behavioural development comes in one of two forms. One is work on the specialization of the two cerebral hemispheres and the other is information about groups of children who have

specific difficulties, such as problems with reading, which might be due to some malfunction in the central nervous system. The predominance of these two sources of information is clearly reflected in the articles in this new journal. Several deal with the different functions of the left and right hemispheres and the possibility that the relations between them might change with age. Many of the remaining papers are about problem groups of children. The opening article is about dyslexia, and others deal with learning difficulties of one sort or another. There are as well papers which do not fall into these two categories. Several deal with old age, and there are some worthy, though speculative, attempts to relate developmental changes in behaviour to neuroanatomical changes.

By and large the standard is high, though there are lapses. It ought to be editorial policy by now to be wary of experiments which simply compare groups of backward and normal readers whose age is the same and who thus have different reading levels. The trouble, now widely recognized, with this sort of comparison is that it cannot distinguish cause from effect. A difference between the two groups could as well be the result of their backwardness. Yet results of this type are reported in the journal without any mention of their inherent weakness.

In general, though, the journal is competent and interesting. It provides a useful extension to the platform already built by *Developmental Medicine and Child Neurology*, and it deserves the attention of psychologists and neurologists alike. □

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Distinct potential

D. Aled Jeffreys

Electroencephalography and Clinical Neurophysiology: Evoked Potential Section. Editor-in-chief Hans van Duijn. Elsevier. 6/yr. \$83 (institutional); \$55 (individual).

REPORTS of evoked potential studies appear in a wide variety of journals, with the choice of journal usually reflecting the particular aspects of applications being studied. Thus, evoked potential investigations of sensory function are commonly submitted to, say, *Experimental Brain Research*, *Audiology* or *Vision Research*, whereas more psychologically orientated work is likely to be reported in, for example, *Psychophysiology*. A favoured target for clinically related papers, on the other hand, has long been "The EEG Journal" (*Electroencephalography and Clinical Neurophysiology*), and it was in response to the steadily increasing proportion of such contributions that this specialized section was introduced in 1984. It is the first publication devoted solely to this topic but, despite the distinct title *Evoked Potentials* printed on the cover, it remains a constituent part of "The EEG Journal".

According to the introductory editorial, this additional section is devoted to clinical and experimental evoked potential research, but the emphasis is, and probably will continue to be, clinical. In fact, one reason given for maintaining *Evoked Potentials* as a part of its parent journal was "to demonstrate that the area of evoked potentials is, and should remain, an integral part of clinical neurophysiology". An additional reason for introducing this specialized section was to "increase accessibility to workers in related fields whose interest in clinical neurophysiology is largely confined to evoked potential phenomena". This aim is partially offset, however, because several evoked potential papers continue to appear in the non-specialized volumes, and the criteria for allocating papers between the different sections are unclear.

The Instructions to Authors are fairly standard. Papers may be in either English or French, but all so far published have been in English: however, abstracts must be submitted in both languages. Both long and short articles are accepted, and they must be accounts of original investigations. There are occasional book reviews and regular advertisements for equipment, but, in general, no editorials, review articles or comments on previous papers. The journal is well produced on good quality, but uncomfortably glossy, paper. The delay from acceptance to publication of most long papers is 6–7 months,

and for short communications about 4 months.

Even though the proportion of evoked potential papers in "The EEG Journal" continues to rise, there is no firm evidence that the introduction of *Evoked Potentials* has as yet either extended the scope of its coverage or diverted many papers from going to other less-specialized journals. Nevertheless, this new section provides a convenient focus for clinically orientated evoked potential papers and will probably continue to gain in importance in relation to its companion volumes. □

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Putting the heat on

Paul Carnochan

International Journal of Hyperthermia. Edited by S.B. Field, J. Overgaard, G. Hahn and T. Sugahara. Taylor & Francis. 6/yr. £80, \$144.

IN RECENT years, the investigation of hyperthermia for the treatment of cancer has graduated from an empirical and largely anecdotal subject to one supported by a growing volume of experimental data. In common with many new branches of science "hyperthermic oncology" draws upon the resources of a wide range of disciplines, creating difficulties both in the communication of new ideas and in gaining efficient access to the relevant literature. The emergence of the *International*

Journal of Hyperthermia is therefore appropriate. The journal has a truly international character, with an editorial board representing 14 countries and, thus far, a clearly non-parochial content.

The editors have aimed to attract everyone with a serious interest in the subject, and the variety of contributions to date suggests that every effort is being made to maintain a wide readership. The four issues available for review are of a uniformly high standard, showing a good balance between invited review articles and original research papers, and covering the whole spectrum of relevant topics from the subtleties of heat-shock protein synthesis through to the clinical front line. I hope that this diversity in subject matter will be sustained and that, in time, proportionally more clinical studies will be represented, reflecting what should surely be the ultimate goal of research effort in this field.

A notably short publication delay of 2–3 months appears to be the norm following acceptance of submitted material, with roughly the same time being taken for refereeing and revision. Overall production standards are high and the choice of a small format with full-width illustrations has ensured excellent clarity.

The journal's long-term prospects will depend upon either the clinical success of hyperthermia in cancer therapy or the editors' ability to attract material from the wider field of related medical topics. Judged purely on the quality displayed so far, however, it clearly deserves to succeed. □

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Choice of delivery

Malcolm Moore

Journal of Controlled Release. Editors Jan Feijen and Jorge Heller. Elsevier. 9/yr in 3 volumes. Dfl. 750.

Journal of Microencapsulation. Editor J.R. Nixon. Taylor & Francis. 4/yr. £50, \$90.

THESE two journals have a lot in common. Both were founded in 1984, have international editorial boards and are published in English; in both, the United States is the most frequent, but not predominant, source of contributions. Their editorial policies imply a greater breadth of coverage than is evident in recent issues, but in this respect editors are at the mercy of contributors and inevitably papers from currently active areas of research are in the majority. At the moment, their coverage overlaps significantly.

Journal of Controlled Release deals with

the control of the rate or site of release of active agents by any means — chemical, physical or mechanical. Nearly all of the contributions are pharmaceutically orientated, and over half of them are concerned with the preparation or properties of gel or polymeric matrices, ranging in size from microspheres upwards, and their ability to entrap drugs and release them by diffusion or after erosion. Others deal with drug transport across skin, degradable drug-polymer conjugates and emulsion systems. It is disappointing that only one among them is specifically concerned with targeting.

Papers are generally substantial in length and of good quality. Acceptance usually takes several months, and is often granted only after revision which suggests a Rhadamanthine editorial board. Besides full papers there is news from the Controlled Release Society, of which this is the official journal, a calendar of relevant meetings, a review of US patents and an occasional section of book reviews. The quality of print is good, though the

glossy paper, while durable, is unpleasant to the touch.

Journal of Microencapsulation aims to cover the preparation and applications of microcapsules, liposomes, nanoparticles, microcells and microspheres, and the physics and chemistry of both wall and core. About half of the papers deal with microcapsules, and another quarter with liposomes. Most discuss the preparation and characterization of capsules or the entrapment, release and effects of pharmaceuticals. There are some papers of general interest on liposome permeability or breakage which could more appropriately



have been published elsewhere.

The standard of the contributions is variable — a few are rather thin in content — but delays between receipt and acceptance are in terms of weeks rather than months. Short communications, and occasional reviews and editorials, are also published. There is, too, a section of patent abstracts, while literature alerts make up about a quarter or more of each issue. Here, the catholicity of reference reflects the breadth of coverage promised in the editorials. The journal is well printed on a semi-matt paper that does not preclude the perfectly adequate reproduction of half-tones, and it is a pleasure to handle.

I assume that journals such as these are acquired as well as, and not instead of, other publications. They will be most useful if they are directed at a well-defined interest group and provide detailed coverage of methods and applications unlikely to be found elsewhere. *Journal of Controlled Release* successfully achieves both ends. Started as a quarterly, it now appears nine times a year yet each issue still contains seven or eight papers. There seems to be sufficient interest in the field for it to thrive.

Journal of Microencapsulation has rather broader ambitions and hence a less obvious role. It could usefully narrow its scope and concentrate on the applications of microencapsulation, which may be the only interest that liposome and microcapsule specialists have in common. The most recent issue I have read contains over six pages of patent abstracts and 18 of literature alerts, but only four full papers and one short communication. Unless the proportion of original material increases, potential readers will find many issues contain little to interest them but the abstracts and alerts. These are quite useful but I doubt that they alone would provide a sufficient reason for libraries to take out a subscription. □

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Tests of the times

Michael D. Rawlins

Pharmaceutical Medicine. Editor Robert N. Smith. Macmillan, London. 4/yr. UK £48, North America \$99, elsewhere £68.

DOCTORS working in the pharmaceutical industry practise a very different type of medicine from that of their clinical colleagues. They are intimately involved in the pre-marketing development and post-marketing surveillance of their companies' products, but unless they carry on a part-time clinical practice they do not personally prescribe their drugs for patients. They tend to suffer a degree of professional isolation, and it is therefore understandable that they should have established their own speciality of pharmaceutical medicine. Indeed, in Britain they have their own professional association, and a diploma which is awarded jointly by the Royal College of Physicians of London, Edinburgh and Glasgow. A journal was the next obvious step.

Pharmaceutical Medicine attempts to provide "an international journal for the critical assessment of new and old methods employed to test drugs in man". It also contains editorials and commentaries on subjects of special interest to pharmaceutical physicians, as well as book reviews and correspondence. In addition there is an extensive abstract section containing summaries of papers from a wide selection of specialist journals.

Much of the journal contains material that pharmaceutical physicians, and

others, will find useful. But despite this something has gone badly wrong and two observations, superficially unconnected, go some way towards explaining what has probably happened. First, although the journal is advertised as a quarterly only three issues have appeared since December 1984 and, by mid-1986, the first volume had yet to be completed. Second, of the eleven original papers that have so far been published, only two have been written exclusively by pharmaceutical physicians. Six have originated from academic institutions, and the remainder (three) have a joint authorship of academics and physicians from the pharmaceutical industry. The papers themselves range from the good, through the impenetrable, to the bad.

I deduce from this that the editors are short of suitable scientific material, and that pharmaceutical physicians are publishing their research findings in other journals such as the *British Journal of Clinical Pharmacology* or its European counterpart. It suggests, moreover, that pharmaceutical physicians prefer to publish their original work in more broadly-based journals. If this is the case, the outlook for *Pharmaceutical Medicine* is poor: it will either become a purely "trade" magazine, or it will disappear. In either event I would have considerable sympathy for the editorial board and the publisher. Failure should reflect not on them, but on the lack of support from their professional colleagues. □

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How interesting?

Daniel W. Foster

Diabetes Research and Clinical Practice. Editor-in-chief S. Baba. Elsevier. 6/yr. Dfl. 305.

Diabetic Medicine: Journal of the British Diabetic Association. Editor John Ireland. Wiley. 6/yr. UK £60 (institutional), £45 (individual); elsewhere \$110 (institutional), \$82.50 (individual).

Two relatively new journals devoted to diabetes and related research have joined the steadily expanding group of publications covering this subject. *Diabetes Research and Clinical Practice* is associated with the International Diabetes Federation and is published in collaboration with the Western Pacific Region of that organization. Its pages include editorials and letters to the editor, but primary emphasis is on original articles describing clinical and basic investigations. Epidemiological research is well represented, and many of

the papers derive from Asian or Western Pacific institutions.

Diabetic Medicine is published by the British Diabetic Association and includes a broader array of articles. In addition to original contributions, editorials and letters, it includes reviews, technical notes, educational advice, economic studies, comments on social issues and abstracts from scientific meetings of the Association. The emphasis is heavily clinical, and in the four issues reviewed only one article could be classified as basic research. By contrast, the four issues of *Diabetes Research and Clinical Practice* had ten articles describing basic investigations. Both journals are attractively and professionally produced.

Evaluation of new journals is difficult because, like physicians and scientists, they can mature and grow in importance. The question at hand, however, is not future performance but current value. Perhaps the best measure of scientific importance comes from the "impact factor" calculated and published by the editors of *Current Contents*. The journals under re-

view are too new to be evaluated by this process, so in its place I made my own, rather simpler, assessment. Every article in the eight issues was reviewed, and then ranked in one of five arbitrary categories: (1) not new, no or minor interest; (2) not new, confirmatory interest; (3) new, no or minor interest; (4) new, significant interest; (5) important. These judgements are, of course, purely subjective, but as a crude control I evaluated four issues of *Diabetologia* in the same way (*Diabetologia* was chosen because it is an international journal published outside the United States and because, as its former editor, I thought that *Diabetes* should be excluded).

The results are shown in Table I, and on this basis *Diabetologia* emerges as a con-

Table I Percentage of individual articles in each category

"Interest factor"	1	2	3	4	5
<i>Diabetes Research and Clinical Practice</i>	48	10	29	13	—
<i>Diabetic Medicine</i>	33	31	19	15	2
<i>Diabetologia</i>	19	19	36	26	—

siderably stronger journal than either of the new entries: it publishes less that is not new and more that is of significant interest. This is hardly surprising — one is comparing an established publication to new journals. Both the new publications contained a few articles of significant interest, however, reinforcing the view that worthwhile material can appear in any journal no matter what its overall standing in the scientific community. The distributions shown in the table also support the view that much of what is published, even in the best journals, is of marginal value or frankly trivial. Only one paper, an invited lecture, was judged important in the current assessment.

Overall, I consider that both of these new journals should be carried in all major medical libraries. They need to be available where research and writing is going on, but I do not think either has reached a position that regional or hospital-based libraries with limited budgets should subscribe. *Diabetes Research and Clinical Practice* will surely be a source of pride to physicians and scientists in Asia and the Western Pacific, and is worth the subscription price to such individuals. *Diabetic Medicine* will be especially useful to practitioners of medicine and will probably have increasing readership in Britain and Europe. For the present, however, these two journals do not fall into the category of "must read", even for specialists. Time will tell whether either can move into that category. □

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The alternatives in radical research

Graham Burton & Danial Wayner

Free Radical Research Communications. Managing editor J.V. Bannister. Harwood. 6/yr. UK £154 (corporate), £124 (institutional), £61.60 (individual); North America \$184 (corporate), \$148 (institutional), \$73.60 (individual).

Journal of Free Radicals in Biology & Medicine. Editor-in-chief Kelvin J.A. Davies. Pergamon. 6/yr. UK £75, North America \$125.

THE free radical, once an almost exclusive property of chemists, has now become the object of much interest from biologists and medical researchers. The reason behind much of this new activity is the suspected involvement of free radicals, especially oxy-radicals (hydroxyl, superoxide and lipid peroxyl) in the onset and development of diseases such as cancer, as well as in some conditions or effects associated with other diseases (for example inflammation in arthritis, and tissue damage with reperfusion after a stroke or heart attack).

Until recently, the only real foci of communication between scientists in this interdisciplinary field have been symposia and conferences, and relevant articles have appeared in various and widely scattered journals. Within the space of a year, three specialist publications have appeared in response to this problem. Although this might seem to be overkill, each journal has tried to carve out a particular niche for itself.

Free Radical Research Communications emphasizes the rapid publication of "emerging ideas and developments through significant research papers and hypotheses as well as short communications and reviews". Despite the rather general title of the journal, and the intention to publish articles on the chemistry of free radicals (a function already served well by the existing chemical literature), the articles published in the four issues

ers, others had no date of receipt or acceptance, and one 24-page paper had neither an abstract nor a summary). Possibly, too, it is a consequence of lack of rigour in the review process (the name of the editor accepting the article is given at the end of nearly every paper, which reveals that more than two-thirds of the papers were accepted by just two of the 15-member editorial board; also, there is no indication of manuscripts being subjected to revision prior to publication). The number of articles per issue has ranged from five to eight, and among them has been



one short review and two methods papers. There have been several book reviews but no letters. The standard of production is good.

Journal of Free Radicals in Biology & Medicine appears to have been conceived with more thought to its purpose — it is better organized, more focused and has a more consistent and higher editorial standard. The stated aim of the journal is to publish articles "dealing with all aspects of free-radical production, metabolism, damage, protection and measurement in biological systems" in the form of peer-reviewed "original contributions" and the curiously named "hypothesis papers"; these are "current, state-of-the-art reviews of significant frontier areas", a designation which seems intended to encourage authors to venture provocative and stimulating suggestions for the purpose of engendering healthy controversy.

In the three issues reviewed there were from eight to eleven articles per issue. These included five reviews and two methods papers. The original articles are full papers which, for the most part, are well-written and provide ample detail in the experimental sections. The articles are well laid out and the standard of production is high. There were several book reviews, but no letters, and there is a useful calendar and notices section.

The articles in both journals deal with essentially the same subject material. At this stage, the *Journal of Free Radicals in Biology & Medicine* is having more success in meeting its stated goals and, in so doing, is best serving the field.

The third new journal, *Advances in Free Radical Biology and Medicine*, edited by William A. Pryor and published by Pergamon, is devoted to publishing in-depth reviews. The first issue appeared too recently for formal inclusion in this review. □

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Free Radical Research Communications

reviewed are concerned almost exclusively with the other stated areas of interest: "the production of free radicals by xenobiotics and biological systems, free radical damage to cells and tissues and defence mechanisms against free radical damage".

The quality and length of the articles is quite variable. This appears to result from an unevenness in the editorial approach (abstracts were missing from several pap-

Multiplication in specialization

Julian Davies

Journal of Biotechnology. Managing editor A. Fiechter. Elsevier. 12/yr. Dfl. 861, \$347.

Biotechnology Progress: A Journal of Food, Pharmaceutical and Bioengineering. Editor M. L. Shuler. American Institute of Chemical Engineers. 4/yr. US \$50; elsewhere \$55.

MIRCEN Journal of Applied Microbiology and Biotechnology: A Research Journal for Biotechnology in the Developing World. Editors J.C. Senez and F.A. Skinner. Oxford University Press. 4/yr. UK £20; North America \$45; elsewhere £25.

THERE are now many journals and review series devoted to biotechnology, a specialization which is apparently being strengthened by the attitudes of editors of the more established scientific journals who narrow perspectives by not accepting papers that are "too applied, too technical, too commercial etc". Comparatively few publications attempt to present a broad spectrum of biological sciences these days.

Given this situation of exclusivity, it is not surprising that specialized biotechnology journals have mushroomed. Provided that they maintain good scientific standards with peer review, their presence is welcome and fills an important need. Within biotechnology journals there is also specialization with respect to subject area and, obviously, readership. These three recent additions to the roster differ greatly in philosophy and level of scientific acceptability, but they would all appear to fill a need.

Of the three, the *Journal of Biotechnology* has the most expansive approach and is produced for profit by a well-known scientific publisher. It is also the most expensive: at nearly 12 cents (US) a page its price is inflated compared to many of the more popular scientific journals (*PNAS*, *Nature*, *Cell*, *EMBO Journal* all work out at about 5 cents per page). *Journal of Biotechnology* extends its outlook by providing useful reviews (on protein secretion systems, for example) as well as original articles. The review period is surprisingly short, and is often as little as 4 days!

In its instructions to authors, the journal makes an unusual comment: "fermentation has become a very ambiguous expression very popular in English-speaking countries. Therefore it should not be used in this journal". Why such censorship? Is "fermentation" now a naughty word?

Biotechnology Progress is a publication of the American Institute of Chemical Engineers and is one of the most specialized

of the available biotechnology journals, being directed towards engineers solely, with many technical illustrations. It contains much technology and almost no biology; it is exclusively concerned with the problems of reactors, separation techniques, downstream processing and so on, and the papers are of a high standard and well-presented. No review period is indicated. It is *not* an international journal — in the issues examined, all the authors were at institutions in the United States. This is not the case for what is probably the leading journal in the field, *Biotechnology and Bioengineering*, published by Wiley, which also has a much broader purview.

MIRCEN Journal of Applied Microbiology and Biotechnology is published for UNESCO by Oxford University Press. It is a research journal devoted to topics relevant to the needs of the developing world and covers all aspects of the practical applications of biotechnology: food, medicine, environment, energy, waste, insect control etc. Included are occasional review papers pertinent to its

brief, for example on mycobacterial disease; in this article it is pointed out that there are ten million new cases of TB every year and some three million deaths worldwide. (Why doesn't TB get the same attention as AIDS?) This journal serves as a means of disseminating UNESCO and other resource information, which may in fact be its major utility; its scientific base is broad and this journal would not attract research papers of state-of-the-art urgency.

It would seem that the available biotechnology journals now cover all experimental aspects of the subject, from the killing of flies in toilets in West Africa with bacterial toxins, to new expression vectors, to designing multimembrane bioreactors. Whether there is justification for continued multiplication in this area is debatable, since as evidenced by these journals the fruits become less and less tasty. □

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Sense of biology

A. Martin Smith

Biosensors: An International Journal. Managing editors I.J. Higgins, W.G. Potter and A.P.F. Turner. Elsevier Applied Science. 6/yr. UK £80; elsewhere £88.

THE area of research and development which has become known as "biosensors" draws upon a very broad range of disciplines, from physics, electrical engineering and electrochemistry, through to microbiology, biochemistry and immunology. In consequence publications in this area have been spread across a number of journals, and there has been no single journal devoted to the subject.

Biosensors seeks to rectify this problem. The introductory editorial states: "We intend that *Biosensors* should provide a focus for review articles, informed comment, original papers and news items". Furthermore, the definition of biosensors chosen is deliberately loose and includes optoelectronic as well as electrode devices, and the initial articles have included a paper on the use of quadrupole mass spectrometry in the monitoring and control of fermentation.

At least in the first four issues, the emphasis has been on relatively long review articles giving a comprehensive grounding in the main areas of current biosensor research. Topics covered include basic concepts, the estimation of biomass, amperometric sensors and optoelectronic immunosensors. More specific articles on urea and glucose

measurement electrodes, and sensors for hydroponics have also been published. The success of biosensor devices depends ultimately on the ability of industry to make sensors with reproducible and reliable performance, and at a price which allows viable commercial use. This need has been recognized explicitly in one of the papers published and it is to be hoped that further articles will expand on this theme in specific sensor areas.

The journal is of small format (165 x 240mm) but the print size is relatively large and clear, as are the reproductions of figures. The editors' declared aim is that *Biosensors* should be international, and that as it matures there should be more



original papers, news items, book reviews, conference and meeting reports, and letters to the editors. The journal can certainly claim to be European but no articles from the United States or Japan were published in the first issues, although influential researchers from both of these countries are represented on the editorial board. Similarly, only articles have been published so far. The ultimate success of the journal will depend on it expanding its scope and in this respect it certainly deserves to succeed on the basis of the quality of the initial papers. □

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Keeping up with the latest trends

David Cove

Trends in Genetics: DNA, Differentiation & Development. Editor Alison Stewart. Elsevier. 12/yr. Dfl. 500 (institutional); Dfl. 150, £33, \$57 (individual).

THIS journal follows the traditions of excellence which were rapidly established by its sister journal, *Trends in Biochemical Sciences*. The principal content of each issue is a number of brief, commissioned reviews of topics of current interest in genetics. As its subtitle suggests, most of the reviews are in the molecular and developmental areas, but the scope of some is a good deal wider; in particular, the evolutionary implications of recent findings get deserved emphasis.

The reviews show a consistently high standard of writing, are commendably up to date and, in general, are similar to the equally high-quality reviews which appear in *Cell*. Each is only a few pages long, and therefore necessarily limited in scope, but most give references which quickly lead the reader to further, more detailed or broader articles. They are therefore ideal both for established scientists, to help them keep abreast of developments in fields distant from their own, and for students where they will provide a way into topics that are to be studied in greater depth.

In the "Perspectives" section a number of articles have appeared which are midway between reviews and essays. These allow the author more licence to put forward personal ideas, and I found some of them very stimulating and enjoyable. A limited number of papers, dealing with historical aspects of genetics, appears under the heading of "Retrospectives". Perhaps it is a sign of my age, but I enjoyed these too, particularly a two-part article on Thomas Hunt Morgan.

Each issue also contains sections entitled "Monitor", which has much the same aims as "News and Views" in *Nature*, although it of course concentrates on



genetical and developmental topics, and "Technical Tips" which provides an opportunity for the publication of (unsolicited) information on experimental techniques. Finally book reviews, correspondence and "Genetic Jottings" are included, the latter providing a notice board for information on courses, meetings and workshops.

This journal should prove invaluable to all students and researchers in molecular and developmental genetics. It can also be recommended to non-specialists, as the reviews attempt to keep to a minimum the amount of background knowledge required to understand them. □

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Down to earth

Richard G. Burns

Soil Use and Management. Editor A. Wild. Blackwell Scientific. 4/yr. UK £37, North America and Japan \$72, elsewhere \$44.50 (institutional); UK £28, North America and Japan \$55, elsewhere £33.50 (individual).

Biology and Fertility of Soils. Editor-in-chief J.C.G. Ottow. Springer-International. 8/yr in 2 volumes. DM 490.

I'M SURE that I am not alone in experiencing a sinking feeling whenever a new journal is announced. It is difficult enough keeping up with the existing literature without the addition of yet another batch of papers. Despite such misgivings fresh contenders for a slice of library budgets continue to appear, and 1985 saw the debut of two quarterly, A4-size journals in the general area of soil science.

The first, *Soil Use and Management*, is a British Soil Science Society publication edited by Professor Alan Wild at the University of Reading. Its intention is to com-

plement the Society's established *Journal of Soil Science* by publishing papers that are of direct relevance to applied agriculture and crop productivity. With the exception of a few articles that would be suitable for, say, the *Journal of Agricultural Science*, there is no obvious home for many of the papers appearing in the first issues. Indeed many of them might previously have been buried in reports and bulletins of limited circulation.

The first four issues are thematic and deal successively with acid rain, upland soils, assessment of nitrogen fertilizer requirements and soil erosion. No one can doubt the practical significance of these topics to world agriculture. Subsequent volumes will deal approximately equally with specific and general topics. Some issues also have a short news section — most valuable when devoted to a succinct overview of a particular subject.

The quality of science and presentation of the 7–12 papers per issue is good, although I'm not convinced that the editor is receiving enough material to allow a critical selection that is independent of the need to fill each issue. While this must be a concern during the formative volumes of

most journals, I'm more worried that the readership of *Soil Use and Management* will be limited by a decision to restrict the journal's emphasis to temperate-zone agriculture. This is reflected in the papers, the vast majority of which come from Britain. I would like to see the journal becoming more international in its coverage and encouraging papers on such topics as arid zone and tropical agriculture. In many ways British agricultural science has been too successful for its own good (witness the drastic surgery now being applied to AFRC institutes) and it needs to look outwards and act as a guiding influence in Third World agriculture. I hope that *Soil Use and Management* will evolve to be a force in this. For the moment, however, the journal has made an encouraging if rather introverted start.

Biology and Fertility of Soils is a rather different publication. When it was first mooted most soil biologists that I spoke to couldn't conceive of a niche for it since the subject matter is exactly of the type covered by two established high-quality journals, *Soil Biology and Biochemistry* and *Plant and Soil*. A few cynically suggested that it might prove to be a dumping-ground for rejected papers. However, for prospective contributors new journals often have an important advantage over established ones in that they publish comparatively rapidly (not particularly fast at around 6–8 months for *Biology and Fertility of Soils* but still somewhat better than its older rivals). The editor, J.C.G. Ottow of the University of Hohenheim, Stuttgart, has collected a large and august editorial board and thus ensured an early supply of papers from famous name authors — these are generally not throw-aways either, but good and thought-provoking articles.

The journal encourages papers in four overlapping areas: soil biology, soil fertility, soil hygiene and soil biotechnology, and has a strong international flavour with some 14 countries being represented in the first volume. There are about eight original papers per issue and some reviews are promised. A future issue will be devoted to a single topic, namely the effects of inoculation with associative nitrogen-fixing bacteria on plant growth: a stimulating and controversial subject if ever there was one! The quality of production is high with the notable exception of the figures, many of which are poor — tighter control is needed here.

Overall, *Biology and Fertility of Soils* has surprised its doubters by making an auspicious start, and I'm sure that many departments will be looking for ways to subscribe to it. In the meantime it will be a frequently-requested item through inter-library loans. □

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Society prospects: on one hand . . .

Brian Whitton

FEMS Microbiology Ecology. Editor-in-chief H. Veldkamp. Elsevier. 6/yr. Dfl. 275, \$111 (institutional); Dfl. 136, \$55 (individual).

MICROBIAL ecology is one of the faster-growing branches of microbiology. Up to quite recently, however, most papers on the subject were scattered through the mainstream microbiology or environmental journals, some of which welcomed them and some of which accepted them only with reluctance, and were also to be found in a variety of symposium-type books. But there is an increasing tendency to publish relevant work in journals largely or entirely devoted to the subject, and *FEMS Microbiology Ecology* is a recent addition to these.

The stated aims are to deal "with fundamental aspects of the ecology of micro-organisms in natural soil, air or aquatic environments, or in artificial or managed environments". So far, the journal has done well in satisfying these interests — the articles have covered a wide range of heterotrophic and autotrophic organisms

from many different types of environment, and collectively demonstrate that microbial ecology has become a truly quantitative branch of science. Most papers have come from Europe, with a relatively large proportion from The Netherlands, partly no doubt because of contacts with the editor but also because microbial ecology is better represented there than elsewhere in Europe.

Publication is rapid, about three to four months from the date the paper is accepted, 50 free reprints are offered and there are no page charges. Editing, general lay-out and figures are all of a high standard, though the FEMS system of using one series of volume numbers for their three journals is likely to cause confusion in the library. For instance the two most recent *FEMS Microbiology Ecology* volumes are 31 (1985) and 38 (1986); during 1986, volumes 33–37 are *FEMS Microbiology* and 39 is *FEMS Microbiology Reviews*.

The quality of the journal is such that it deserves to succeed. The only doubt is



that a publication serving such a broad field, and with numerous contributors competing to get high-quality papers accepted, probably needs to issue much more than one volume each year if it is to become a prestige title. My guess is that the journal will eventually either have to expand, in order to compete with the American Society for Microbiology's *Applied and Environmental Microbiology*, or select some aspect on which to specialize. I hope it proves to be the former. □

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. . .but on the other

David H. Boxer

FEMS Microbiology Reviews. Editor-in-chief G. Gottschalk. Elsevier. 4/yr. Dfl. 275, \$111 (institutional); Dfl. 149, \$60 (individual).

FEMS Microbiology Reviews was launched by the Federation of European Microbiological Societies with the intention of providing profound and readable reviews of progressing areas of microbiology. The appearance of a European-based review journal will be widely welcomed, but this publication does not seem to be destined to become an authoritative alternative to the American Society for Microbiology's *Microbiological Reviews*.

The journal is well laid out in the usual FEMS format, but would benefit from clearer illustrations. The articles themselves are quite readable with most, but not all, carrying a useful summary. I found the quality rather variable, however. Some articles are genuinely authoritative and review topics of wide importance, but many others are far too short and insubstantial. For example, one of them is a mere four pages long, and over a third are of eight pages or less. Another contains 15 figures and one table of one of the authors' unpublished observations which surely ought to have been published in the primary literature rather than a review journal. It is difficult to equate brevity with profundity, and many of the shorter articles may have found a better home in the more popular *Microbiological Sciences*.

Despite the short time, usually a few weeks, taken for an editorial decision, the publication as a single issue of the last two numbers of the most recent volume clearly led to long publishing delays, in one case some ten months. But few journals can claim to correct printing errors as fully as this one, since in one case it republished a complete article in order to set matters right!

A comparison with the contemporary volume (49) of ASM's *Microbiological Reviews* revealed that the ASM journal published 20 articles each citing, on average, over 200 publications, to the FEMS journal's 16 articles whose average bibliography lists just over a 100 references. At the institutional price, the FEMS journal is about three times more expensive per cited publication and the comparison is even less favourable at the personal subscriber level. I doubt whether many individuals will consider taking out a personal subscription, and I cannot see its justifying a place in the library unless the quality of its articles becomes more uniformly high. □

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Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1987. This information is not complete in all cases; an individual rate may be available, for example, or there may be an additional charge for air-mail delivery. Readers interested in a particular journal should therefore check prices with the publisher before subscribing.

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Element exchange

R. W. Raiswell

Biogeochemistry. Editor-in-chief Robert W. Howarth. *Martinus Nijhoff*. 6/yr in 2 volumes. Dfl. 346, \$145.

INTERACTIONS between the biological and geochemical cycles of the major elements are a significant area of research with important economic and social implications. *Biogeochemistry* seeks to tap, and thence to integrate, this interdisciplinary and diverse field which is undergoing such rapid development. In this respect few new journals can have been so admirably conceived, with the potential to exercise a role in the vanguard of a discipline.

The first five issues have attracted contributions from a wide spectrum of biogeochemistry, in accordance with the editorial aims of dealing with biotic controls on the chemistry of the environment and with geochemical controls on the structure and function of ecosystems. The breadth of this approach is reflected by papers ranging from terrestrial to aquatic systems (freshwater and marine) and spanning scales varying from the experimental to the global. All but one of the contributions report original research, although review articles and short communications will also be accepted. A further section will contain news, notes and letters to the editor. Last, but not least, are book reviews and announcements of meetings.

The scientific standard of the contributions is at least reasonably good and a

satisfactory proportion of them are of high quality. Further judgement is reserved, if only because of the difficulty of the task the editors have set themselves. For better or worse, practising scientists develop their research interests dendritically, extending into new areas away from a core of more widely shared interests that are usually defined by their training and professional circumstances. That core attitudes, approaches and objectives can be remarkably different is well-illustrated by comparing the treatments of similar problems in the soil science and sedimentary geochemical literature. The comparative simplicity of the latter has encouraged rigorous and quantitative treatments, whereas the complexity of the soil system favours greater pragmatism. Clearly, reconciliation of these approaches would be valuable, although the two will initially find themselves somewhat uneasily confined between the same covers. The development of more refined global models depends critically on linking both approaches to integrate the effects of a multiplicity of different terrestrial systems.

Biogeochemistry is technically more than equal to this task. It is well-produced, attractively laid out and not unduly expensive. Both authors and editors need to give more attention to proof-reading and typesetting respectively, as some of the papers contain errors of omission which are sufficient to obscure any meaning. I hope that these are teething troubles, because this journal deserves to be successful. □

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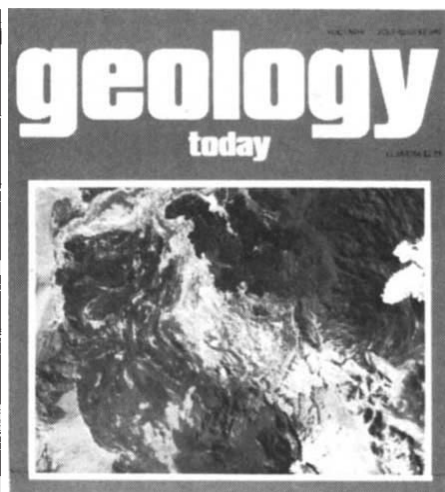
Top of the rocks

Paul Mohr

Geology Today. Editor-in-chief. John H. McD. Whitaker. *Blackwell Scientific*. 6/yr. UK £45.50, North America and Japan \$87.50, elsewhere £54.50 (institutional); UK £15, North America and Japan \$28.50, elsewhere £18 (individual).

TIMES have changed since Adam Sedgwick talked geology to over 3,000 people on Tynemouth beach in 1838, and Roderick Murchison was lauded before an even greater audience in 1849 by the Bishop of Birmingham with the words "Hail, King of Siluria". Through the intervening period, the science of geology has become specialized to the extent that the specialists are hailed, if at all, only by a handful of peers. The place of geology among the sciences, and public awareness of it, have shrunk, as secondary educational curricula around the world bear mostly silent witness.

Geology Today aims exuberantly and confidently to reverse this trend. John Whitaker and Peter Smith (the scientific editor) seek to awaken the public, and alert some professionals, to the kaleidoscopic content and the vigour of geology. Their menu is a lively mix of articles by geologists who can write straightforwardly (with perhaps a little editing), and concurrent series of items that include an itinerary to an attractive geological locale in Britain, what a particular geological museum offers to visitors, the make-up and life-style of some fossil animal, the symmetry and sources of a particular mineral species, a biographical sketch of one of Britain's pioneers of geology, news and comment, book reviews, and readers' opinion and dissent. The articles and most of the features are illustrated with diagrams of exemplary aptness and clarity, and sharply-reproduced photographs embellish the spectacular with an artist's eye for beauty. To aid the general reader's digestion, the margins of the main articles are used for succinct definitions of any technical terms that might be unfamiliar.



The flavour of the magazine can be conveyed in a selection of article topics: the Himalaya, Carboniferous crustaceans, ecclesiastical petrology, events across the Precambrian–Cambrian boundary, the blueschist enigma (subtitled "How to Stay Cool under Pressure"), oil in Chalk, Antarctic geology and mineral resources, and the lying stones of Eibelsstadt. Additional, "Geodigest" articles provide pithy ruminations on the more interesting discoveries tucked away in technical journals. "News and Comment" serves up (to take the three items from just a single issue) a century and a half of glacial theory, appeasement in geological politics and electric pterosaur takes off.

One unfortunate impression given by *Geology Today*, and sure to be remedied soon, is that geology is essentially an Anglo-American enterprise. This impoverishes a science that owes much to the energy and ideas of men and women from Germany, Italy, France, Scandinavia, the USSR and so on. Perhaps the editors might have the courage to publish an occasional article in a major language other than "the common tongue of a cultureless commercial world" (to quote a contemporary British poet).

There are lodes in *Geology Today* which will be worked out in a few years: for example, the number of British geology museums is finite. But the editors have zeal and imagination enough to prospect for and discover new ores. Might there yet be a bishop to proclaim to even vaster audiences than those of Sedgwick and Murchison, "Hail, King of Pangaea"? If that seems unlikely, *Geology Today* has nevertheless launched on a pathfinder's role in renewing public awareness of the extraordinary physical setting in which, unbidden, we find ourselves. Not merely universities, but every secondary school and public library should make it available, and aid renewal of a sense of wonder. □

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Of ticks and mites

Jim Berreen

Experimental and Applied Acarology. Editor-in-chief W. Helle. Elsevier. 4/yr. Dfl. 204.

ACAROLOGY has for some time been the poor sister of entomology, both in relation to its range of published material and in the availability of journals from which to gain an overview of the subject. Elsevier's decision to launch *Experimental and Applied Acarology* was thus a positive move to enable the cross-fertilization of the various experimental approaches that previously were documented only in the proceedings of international conferences.

The editorial intention is to address a broad church, from field studies and behaviour through to toxicology and physiological systems. The restrictions are that manuscripts dealing with taxonomy and morphology will only be considered in experimental and control contexts, and faunistic studies need to be supported by an experimental approach. The initial volume reflected this choice well, with nine papers on the effects of pesticides, seven on field studies and 14 on laboratory investigations. Not surprisingly, there have been more papers on ticks than on any other group, but plant parasites and their predators came a close second.

The editorial board is widely international, and eminent, and this should attract an increasingly diverse and interesting range of papers. Certainly the contributions so far have been generally good to very good in quality, with a few delightful surprises like Knop's description of real behaviour and sperm transfer in a tydeid mite and Cunningham's definitive statement on oviposition and fecundity in the flour mite.

Overall, the presentation is of Elsevier's usual high quality. The reproduction standard of micrographs appeared to be adequate to my non-specialist eye, and I could identify all that the authors intended. Perhaps that is sufficient, even if the tonal range and definition of some of the plates may be rather less acceptable to the authors and more discerning critics.

The practice of printing figures drawn by graph plotters is an obvious mark of efficient progress in publishing. However, the fonts for plotters seem to have been designed by graphic gnomes with little concern for the reader — the difference in legibility between poor plotter and good printed output is so substantial that it demands an editorial reaction. One other, small idiosyncrasy of the production is that narrow figures appear left justified. For graphs this gives the probably valuable impression that they have just hit the page, no doubt hot off the bench. How-

ever for one figure, detailing the sampled sections of an ethnic cow (head (1), ears (2) ... tail (7)!), it gave the impression that the subject was just hobbling back to the bush on the two legs it had left — (4) and (6).

In addition to research papers and book reviews, the journal has also published a short review paper. This is especially encouraging, as review articles in acarology are all too rare and are valuable in stimulating new directions and ideas.

Both editor and publisher are to be congratulated on providing a forum which will undoubtedly increase the value and scope of research carried out on this still neglected yet economically important group of animals. The journal reflects the coming-of-age of acarology, and I hope that the editorial board will maintain their broad zoological perspective. □

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Life in the tropics

Gordon H. Orians

Journal of Tropical Ecology. Editor Adrian G. Marshall. Cambridge University Press. 4/yr. UK £49 (institutional), £19.50 (individual); elsewhere \$99 (institutional), \$39 (individual).

THE present, a time of spreading desertification at low latitudes and of rapid conversion of tropical forests to other uses, is also experiencing an upsurge in interest in tropical ecosystems. Because tropical organisms, communities and ecosystems are so much more poorly known than temperate ones, there remains a need for basically descriptive ecological studies to accompany those with a more hypothetico-deductive approach. The high volume of manuscripts submitted to existing ecological journals makes it difficult for tropical researchers to publish primarily descriptive papers. Moreover, papers on tropical ecology are scattered among those reporting work done at higher latitudes.

To encourage the execution and publication of important descriptive research in tropical ecology, and to make the results of tropical research more accessible to ecologists living in tropical countries, The International Association for Ecology recommended initiating a new journal. Launched in 1985 as the *Journal of Tropical Ecology*, it accepts papers dealing with all aspects of tropical ecology, both "pure" and "applied", and includes both original research and reviews of literature. It is edited at the Institute of Southeast Asian Biology at the University of Aber-



deen, Scotland, with the assistance of an editorial board drawn from leading tropical ecologists on all continents. Long and short articles, notes, notices, announcements of meetings, book reviews and editorial comments are all welcomed.

The first volume has a good balance among papers dealing with different taxa, and contains reports of research at all levels of ecological organization, from studies of single populations to ecosystem functioning, and from all areas of the tropics in both the New and Old Worlds. Already in its first year, the journal has received and published papers by leading researchers in tropical ecology. The papers actually are not noticeably more descriptive than those found in the mainline temperate zone ecology journals; indeed, the journal is already well on the way to becoming a major forum for interchange of ideas about tropical ecology, and all researchers working in the tropics or who attempt to follow the tropical literature will find it essential reading.

The decision was made, largely as a result of urging by people from non-English speaking nations, to accept papers only in English, but the editors have expressed a willingness to put in extra efforts to assist authors for whom English is not a native language. Contributions in the first volume are well-written, and despite the admission of the editor of having distinctive views of what constitutes good English, the unique styles of some of the contributors known to me have survived his editorial pen relatively unscathed.

The journal is attractively printed in single-column format and the quality of illustrations and tables is excellent. There were considerable delays in publishing parts of Vol. 1, the last issue not appearing until mid-1986, and it remains to be seen whether these were the result of the birth pains of a new endeavour or symptomatic of difficulties that may persist into childhood. It is, however, unlikely that the journal will suffer from a shortage of high-quality manuscripts, and I suspect that pressure to expand from the current 100-page issue will soon face the editors. □

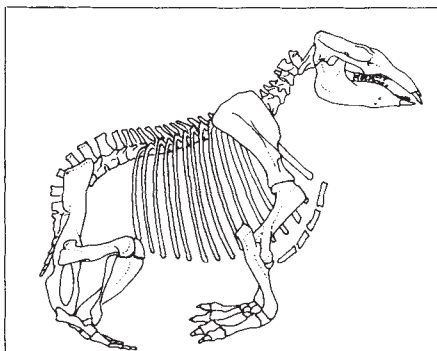
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Zoologists all at sea

Sheila S. Anderson

Marine Mammal Science. Editor Joseph R. Geraci. *Society for Marine Mammalogy*, 1,041 New Hampshire Street, Lawrence, Kansas 66044, USA. 4/yr. North America \$70; elsewhere \$78.

FROM its title, *Marine Mammal Science* might seem to cater for specialists, but it is in fact aimed at a group of people as disparate in their interests as the creatures they work on. "Marine mammals" includes seals, sealions, walruses, whales, dolphins, sea otters, manatees, dugongs and polar bears, creatures which do not all share the same ancestry but have in common a greater or lesser ability to spend time beneath the surface of the sea. While



Bare bones — skeleton of the desmostylian *Paleoparadoxia*, from a paper by L.G. Barnes *et al.* in the first issue of *Marine Mammal Science*.

there are several organizations and individuals who specialize in marine mammal studies, the fascinating adaptations of anatomy and physiology shown by the animals attracts terrestrial mammalogists to study their topic on very different species. The field also lures devotees of a quite different character — those who simply love beautiful seals and the great levathans of the deep.

In the past, papers about marine mammals have been spread throughout the journal world, but now the Society for Marine Mammalogy has offered a central place for the results of research and observations. Each of the six issues of *Marine Mammal Science* published so far contains three or four articles and up to two short notes which are subject to peer review. Letters to the editor are solicited, and there are book reviews, notices of new books and an occasional opinion slot, which is an invited contribution on a selected topic. The first two issues contained news, Society business and "Memories" sections, but these have not appeared in later issues.

The Society made its debut in the pub-

lishing world by including in its first three issues review papers presented by the top people in the field at its 1983 Biennial Conference. This provided an impressive list of authors to begin with, but the quality of the other articles is perhaps of greater importance as a guide to the journal's health. The standard is generally high and there is a good mix of material on the two main groups, seals and whales. The only detectable bias (30 per cent of the articles published to date) is towards papers of an anatomical nature which may be too specialized for publication elsewhere.

The key question is whether *Marine Mammal Science* will attract a wide spectrum of original research papers in the future; a journal dedicated to a named group of animals, and with a relatively small circulation (about 900 Society members and subscribing institutions), may have difficulty persuading topic-orientated workers to abandon the well-established, wide-circulation journals. The time from acceptance of manuscripts to publication of 3–6 months is a very

favourable factor at present, but the \$45 page charge might be daunting, certainly to the individual and these days probably to institutions as well. The paper quality is good, allowing a high standard of illustration, and the editor is keeping a tight rein on standard and style.

The journal was initially used to communicate news to Society members, perhaps to meet the needs of non-scientific members, but this has been discontinued and the content is now strongly scientific. Marine mammalogists need to communicate with each other. Let us hope that they can be persuaded to do this at a scientific level in their new journal. If that happens then the rest of the zoological world will have to keep an eye on *Marine Mammal Science* for the exciting developments, and not just the ones hard to publish elsewhere. □

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Fish figures

T.J. Pitcher

Journal of Applied Ichthyology. Editors W. Ahne, K. Lillelund, H.H. Reichenbach-Klinke and K. Tiews. *Paul Parey*. 4/yr. DM 132.

IN MANY developing countries fisheries provide a crucial source of food and valuable employment yet, compared to the well-fed developed nations, there is often a woeful lack of basic knowledge about the biology of the fish stocks. Scientific management of these fisheries, which are characteristically multi-species and often threatened by conflicts between artisanal and industrialized sectors, must be based on such fundamental research.

A particularly welcome aim of *Journal of Applied Ichthyology* is to publish research on fisheries in developing countries, especially on aid-related projects. It was disappointing, therefore, to find that only two of the 28 papers in the six issues available for review dealt with this subject. In fact, overall there is a preponderance of work on the traditional well-worked species of cod, eel, carp and plaice (nine papers) and on the hallowed fishery themes of age, growth, food and migration (eight papers). In the future, it is to be hoped that the editors can do more to actively encourage contributions relevant to the management of fisheries in developing countries, since it is here that the new journal could meet a need not fully satisfied by longer-established publications.

The journal aims to publish work in all areas of applied fish biology but, apart

from the scientific basis of fishery management, aquaculture (four papers), pathology, immunology and ecotoxicology are specifically mentioned. Surprisingly, since there is already a specialized journal covering this area, the largest number of papers (ten) is concerned with fish diseases.

Of the third-world fisheries papers, a Seychelles fishery survey using trolling lines reveals a small 2,500-tonne stock of tuna, which could be exploited seasonally on a small scale, while yield analysis for a valuable prawn fishery in Kerala suggests that, for optimum yields, current exploitation should not be increased any further. Other interesting contributions examine

Zeitschrift für angewandte Ichthyologie Journal of Applied Ichthyology

the potential of flounders as a by-crop in the cage-culture of salmon, the cultivation of marine amphipods as food for farmed fish, filial cannibalism by male tilapia in relation to reproductive success, and the results of extensive Baltic cod tagging experiments which have previously appeared only in the lamentable "grey literature" of fisheries.

There are a number of minor irritations which should have been put right by referees or the editors before publication. In general, however, this journal will be welcomed in libraries used by fisheries biologists. □

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Look for the little bit extra

Catherine Geissler & T.A.B. Sanders

Food Reviews International: Production, Processing, Acceptance, Nutrition and Health. Editors Roy Teranishi and Irwin Hornstein. Dekker. 3/yr. \$125 (institutional), \$62.50 (individual).

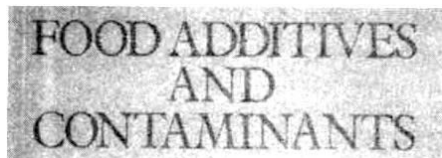
Food Additives and Contaminants: Analysis, Surveillance, Evaluation, Control. Editors R. Walker and M.E. Knowles. Taylor & Francis. 4/yr. £55, \$99.

THE aim of *Food Reviews International* is to bring together "state of the art reviews in food production, food processing and food related disciplines". The journal is "dedicated to those who strive to create a better nourished and healthier world", and to a certain extent the composition of the editorial board justifies the *International* in its title. Most of the board is drawn from Europe and the United States, but while Asia, especially Japan, is also well represented, there is no African member and only one from Latin America.

Only reviews are published. Each issue contains three or four of them, ranging in length from 20 to 70 pages. Speed of publication is not indicated. The standard of production, however, is excellent: good quality paper, with clear print, diagrams and half-tones.

The contributions published in the three issues available for review have had an emphasis on the Third World, several of them dealing with rice. Those in the area of food science and technology have given a lot of detail on chemical composition, and much technical information, while those on food production and nutrition have covered broader economic and social matters. To date the former have predominated, but this might well not be the case in the future.

All of the articles could have been published elsewhere, and what distinguishes the journal is not their specific content but its concentration on the lengthy review



format and the juxtaposition of discussion of all aspects of food, from production through processing to consumption and nutrition. How will it fare? The readership may be limited as it is likely to appeal mainly to food scientists and nutritionists with a particular interest in Third World issues. That is a rather diffuse and limited audience, and the expense of an addi-

nal journal could deter libraries in the poorer countries.

By contrast *Food Additives and Contaminants* has a very well defined constituency. It is concerned primarily with food safety and covers all aspects of additives and contaminants, both artificial and natural, in the food chain. Because of its multidisciplinary nature this journal does indeed fill a gap in the literature.

Short and long papers and reviews are accepted, with a high proportion of them coming from government-funded bodies. Publication time is about six months. Although many of the contributions deal with methodology, and could have appeared in publications such as the

Analyst, the journal also publishes work containing surveillance-type information. The latter, although of great importance, would not be regarded as novel or contributing to the development of new scientific hypotheses by some established journals.

Food Additives and Contaminants is well produced, there are no page charges and the subscription rate is reasonable. Altogether, libraries concerned with food safety and analytical chemistry would do well to have it on their shelves. □

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Biological bytes

Andrew Coulson

CABIOS: Computer Applications in the Biosciences. Executive editors R.J. Beynon and Joseph L. Modelvsky. IRL. 4/yr. UK £55, North America \$95 (institutional); UK £30, North America \$50 (individual).

CABIOS is an A4-format quarterly with about 200 pages per issue. Its intended coverage is adequately described by its subtitle; in practice it is concerned mainly with molecular biology and biochemistry rather than statistics, structure-related computing, clinical applications or population biology.

About half of each issue is taken up with reviews of books and software, news and other ephemeral material. A novel feature is the "literature survey", which consists of abstracts of related papers in other periodicals. At first sight this is useful and impressive, since it is said to be compiled by one person and it refers to a variety of sources, some very obscure. However, there appears to be no mention of the intended journal coverage of this section ("what can I avoid scanning by reading this instead?"). My confidence that the compilation is highly systematic was undermined by finding two different abstracts of the same paper in successive issues of the journal.

The original content of each issue consists of about half-a-dozen full papers, perhaps one half-length "Communication", a (commissioned) review and an elementary review ("First Byte").

Publications in this field are often devalued by three features. The size of the MPU (minimum publishable unit) is about 20 per cent of its value in conventional biological science; there is only a weak taboo on publication of similar material in different journals (or in the same journal at intervals of a few months); and many publications describe implementations of

well-known procedures on particular systems, and contain little that is novel in methods or results. I would award *CABIOS* αβ in avoiding these pitfalls. A very high proportion of the papers in the first year dealt with interesting and useful projects which were otherwise unknown to me, and the reviews have been generally timely and well-done.

However, despite the statement in the Instructions to Authors that "preference will be given to papers that describe new algorithms", the principal novelty in most of these papers is not in the algorithm, but



in its specific implementation. Publications of this kind are almost without value if the programs described are not readily available to the reader. The novel procedure *CABIOS* has adopted is to make it a condition of publication that authors participate in a Reader Enquiry Service (such as many magazines provide for their advertisers) and make software available at no more than a nominal handling charge. Some software in this area has been sold at such outrageous prices that this rule almost deserves to be described as a courageous decision. No doubt it accounts for the absence of some well-known names from the journal.

The format is generally attractive and readable. The lack of copy editing is not without visible effect but it enlivens one's reading to come across a word like "esqueleton" in a paper, and I can certainly tolerate incidents like this or finding several pages bound upside down and backwards if the effect is to keep the price of the journal down. □

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Use of the common denominator

Joel E. Cohen

IMA Journal of Mathematics Applied in Medicine and Biology. Editor R. W. Hiorns. Oxford University Press. 4/yr. UK £54, North America \$138, elsewhere £69.

Communications in Statistics — Stochastic Models. Editor Marcel F. Neuts. Dekker. 3/yr. \$110 (institutional), \$55 (individual).

THE EARLY numbers of the *IMA Journal of Mathematics Applied in Medicine and Biology* have papers (all handsomely printed and illustrated) on human genetics and population genetics, neurobiology, demography, ecology, exploitation of living stocks, epidemiology, parasitology, morphology, chemotherapy, radiotherapy, chemotaxis and human physiology.

What brings these articles together is their common use of mathematical language, just as the common use of English brings a wondrous pot-pourri of scientific topics together under the covers of *Nature*. The mathematical language is as diverse as the subject matter. The first issue alone uses diffusion equations, maximum likelihood, partial differential equations of continuum mechanics, stability theory of non-linear difference equations, computer simulations based on generating functions and matrix theory.

The declared statement of purpose of the new *IMA Journal* is to seek "to stimulate research in which mathematicians are seen to be tackling problems which those in the medical and biological fraternities would like addressed". In its aims and its achievements, it joins a sibship of journals that present mathematical analysis of biological and medical problems. Among these are the *Journal of Theoretical Biology*, *Bulletin of Mathematical Biology*, *Journal of Mathematical Biology*, *Biophysical Journal*, *Ecological Modeling*, *Mathematical Biosciences*, *Theoretical Population Biology*, *Journal of Mathematical Population Studies*, *Computers in Biology and Medicine* and many with a more statistical slant, such as *Biometrics*, *Biometrika*, *Biometrische Zeitschrift* and *Statistics in Medicine*.

A question suitable for discussion in the corridors of scientific meetings is how much these journals influence biology and medicine. It is at least a plausible conjecture that most researchers in biology and medicine are reached and influenced primarily by the mainline journals of their fields. If this is so, then mathematicians who study biological and medical problems will find the new *IMA Journal*, and its siblings, useful for communicating with

one another and for developing their wares to a point where they command the attention and space of the mainline biological and medical journals.

Reproduced photographically from the authors' final typescripts, *Communications in Statistics — Stochastic Models* offers a visual diversity as great as the substantive diversity of the new *IMA Journal*. The emphasis is entirely on the theoretical analysis of models. Articles in early issues deal extensively with queuing theory. Scattered papers discuss reliability theory, self-similar processes, flows in networks with random capacities and parallel processors. A lone stochastic model of cancer metastases represents the only overlap with the *IMA Journal*. No data of any kind appear and numerical illustrations of results are rare.

The mathematical level of the exposition is advanced and sophisticated, as if the authors aspired to *Annals of Probability*, *Zeitschrift für Wahrscheinlichkeit*

stheorie und Verwandte Gebiete, or the Russian journal *Theory of Probability and Its Applications*. The editor's preface emphasizes "the stimulating new problems generated by the modelling of computer and telecommunication systems, of inventories, queues and biological popu-

STOCHASTIC MODELS

lations". If papers in future issues reflect a wider range of these problems, *Stochastic Models* will move away from its purer brethren, mentioned above, towards such journals as *Advances in Applied Probability*, *Stochastics*, *Journal of Applied Probability* and *Stochastic Processes and Their Applications*. □

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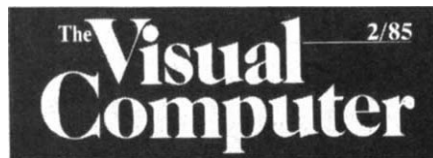
Picture progress

Andrew Dearing

The Visual Computer: International Journal of Computer Graphics. Editor-in-chief Tosiya L. Kurii. Springer-International. 6/yr. DM 337.50.

The Visual Computer is the official journal of the Computer Graphics Society, and is intended to cover rather a wide field, ranging from computer input and perception of visual information, through hardware, software and mathematical tools, to the generation, animation and application of visual images. In common with many societies' journals, it includes useful sections giving "news and views" and an up-to-date calendar of forthcoming events. These, however, are short, and the core of the journal consists of some 6-8 refereed original or technical papers.

Four issues appeared in 1985 and six are planned for 1986. The average length of



an article is about ten pages, allowing authors the chance to write in some depth, and the editors have maintained order and focus by using thematic titles for three out of the four issues in Vol. 1. These covered animation, constructive geometry and the graphics interface, and grouped related articles into single issues in a sensible way without too great a loss of general interest.

During the first year, many of the jour-

nal's intended topics were covered by one or more papers, although there was a stronger emphasis on image generation and output than on input and recognition techniques. A danger in producing a journal whose subject matter includes topics related to the use of visual information is that substance can vanish behind the self-evident quality of the images or the method — shades of the Emperor's New Clothes. *The Visual Computer* suffered from this disease somewhat in one or two contributions that covered particular applications of computer graphics, and the papers in question might well not be acceptable to journals specializing in their own application area.

Otherwise, the journal has a reasonably balanced editorial style, and divides well between application and theory, algorithms, discussion and (yes!) pictures. It contains papers from quite a wide geographical and technical spread of authors, although there is the expected slight bias towards North America.

This is very much a journal to keep readers aware that the discipline has a reasonable breadth, without losing them in undue detail. Most of the papers might find a home in more specialized publications without requiring much modification, but this in itself is not a criticism since *The Visual Computer's* aim is to establish a new nucleus around the whole field of visual information. Does it achieve its intentions? Generally it does, and in a consistently well-presented manner which is attractive and easy to read. But the editors will need to work hard to make the journal cover all aspects of their intended field. □

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Organic decline

M.F. Lappert

C, Molecule Chemistry: An International Journal. Editor-in-chief Igor B. Tkatchenko. Harwood. 6/yr. UK £208 (corporate), £166 (institutional), £83.20 (individual); North America \$250 (corporate), \$200 (institutional), \$100 (individual).

IN THE late 1970s, when there was concern about the depletion of the world's petroleum reserves, a significant amount of research was centred on alternative sources of organic chemicals. A major part of this endeavour was the conversion of synthesis gas, a mixture of carbon monoxide and hydrogen, into methanol, and thence into organic chemicals containing C-C bonds.

The scope of *C, Molecule Chemistry* is restricted to this area of research which, in the past three or four years, has become less urgent. Many large companies are no longer particularly active in the field, and there is no reason to believe that any great increase in research effort is to be expected in the near future. It is perhaps for this reason that although the first number of Vol. 1 of the journal appeared in April 1984, No. 5 was published only as recently as January 1986.

The editor-in-chief, the regional editor and the editorial board members are all well-known practitioners of organometallic and/or catalytic chemistry. So far, in the five numbers available for review, some 30 papers have appeared and many of them have one of the board members among the authors. Hence, the overall quality of the contributions is good, but they could well have been published in better established journals and thus have reached a much larger readership. Most of the papers are long, with substantial experimental sections, but there have also been five preliminary communications and a few book reviews.

The journal is of small format (15 × 23 cm) and the print is attractively produced, with good quality illustrations. The publication times, however, are quite variable; the worst example appears to be that of a paper which appeared in the fifth issue, but which was received in September 1984. Thus far, Vol. 1 amounts to 422 pages, which means the subscription price is on the expensive side.

While I would acknowledge that some useful papers have appeared in *C, Molecule Chemistry*, it is not clear that the journal fills an important gap in the literature. Moreover, the extent of specialization is such that it is unlikely that it will have a good chance of surviving. □

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Under analysis in Japan

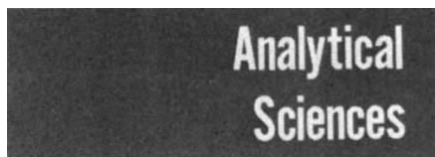
John M. Ottaway

Analytical Sciences: The International Journal of the Japan Society for Analytical Chemistry. Editor S. Fujiwara. Katakura Libri, Inc., 36-9, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan. 6/yr. \$120 (institutional); \$60 (individual).

WHILST the appearance of another general journal in the field of analytical science will no doubt be disappointing to many, this one may well serve a very useful purpose if it encourages Japanese authors to make more of their work available in the English language. *Analytical Sciences* is the new bimonthly journal of the Japan Society for Analytical Chemistry, and to date almost all communications have originated from Japanese authors, and all are in English.

Three categories of article are included, original papers, letters to the editor and "instrumental achievements". The quality of the original papers is, as one would expect, very high indeed, and the subjects covered are very wide with an emphasis on techniques undergoing intense development such as atomic spectrometry, liquid and gas chromatography, electroanalytical methods and flow injection analysis. Average publication times appear to be of the order of five months, with several papers in the April 1986 issue published only two months after submission. Original papers are expected to be six pages or less in length including figures and tables (maximum double-spaced manuscript typescript 18 pages inclusive), whilst the other types of article are restricted to less than two pages.

In the publicity material it is stated that letters to the editor are concerned with opinions of authors working at the forefront of analytical chemistry, and will provide a brief overview of current and future trends, but published articles in this section appear to be mainly shorter papers describing original work. The section called instrumental achievements also



appears to consist mainly of shorter original contributions, but with an emphasis on applications of modern instrumentation and with a preponderance of articles from industrial sources. This seems a particularly worthwhile idea, as too often analytical scientists in industry seem reluctant to publish the excellent work carried

out in their laboratories because of the need to complete a development project in sufficient detail for a full or in this case original paper.

The journal also includes a calendar of forthcoming meetings, which contains a listing of many Japanese meetings as well as the main international conferences. Analytical colleagues outside Japan might find this more useful if more details were included about the Japanese events.

The quality of the English and standard of production throughout the journal are excellent, with beautiful coloured photographs in several issues. With the rapid development of analytical chemistry and instrumentation in Japan, it is perhaps timely that a vehicle allowing wider dissemination and appreciation of the numerous ideas and new technologies should appear. The journal will be essential reading on a world-wide basis, and international authors may find the short publication times, if they can be maintained, very attractive. As yet another new journal to be incorporated into ever-tightening library budgets, it may be difficult to sell, but there is no doubt that the concept and information will be widely appreciated by the analytical community. □

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Surface covering

Douglas H. Everett

Langmuir: The ACS Journal of Surfaces and Colloids. Editor Arthur W. Adamson. American Chemical Society. 6/yr. US \$329 (institutional), \$55 (individual); UK \$342 (institutional), \$68 (individual).

Langmuir was launched in January 1985, with the intention of providing a medium for the publication of both theoretical and experimental work in a wide range of areas of surface and colloid chemistry: solid surfaces in ultra-high vacuum, including surface spectroscopy and structural studies; chemistry and electrochemistry of surfaces; heterogeneous catalysis; all aspects of liquid-liquid and liquid-vapour interfaces; and the great domain of disperse systems. The emphasis, it is stated, is to be upon fundamental science rather than on studies of practical importance. This breadth of coverage is reflected in the name chosen for the journal: few scientists have contributed so widely, nor initiated so many new lines of research in surface and colloid chemistry as did Irving Langmuir.

The new journal has two stated missions: first, to exert a unifying influence to



counter the proliferation of specialized journals in surface and colloid chemistry; and secondly to emphasize that this is a broad, important and established field that merits recognition as such. It will complete the coverage by American Chemical Society publications of the main fields of fundamental chemistry, and in addition to articles and letters will publish expert reviews, the Langmuir Award Lectures and book reviews.

Langmuir is to be welcomed as, in effect, the journal of the ACS Division of Colloid and Surface Chemistry, although it also aims to be an international journal, as shown by the world-wide distribution of its advisory board. It will compete with a number of existing journals, some of which already either claim to cover the same field, or have more general scope but publish a substantial proportion of papers on surface and colloid chemistry. These include in the first category commercial journals such as *Journal of Colloid and Interface Science*, *Colloid and Polymer Science*, *Journal of Dispersion Science and Technology*, *Colloids and Surfaces* and *Surface and Colloid Science*, and in the second *Journal of the Chemical Society*, *Faraday Transactions I*, *Berichte der Bunsen Gesellschaft* and *Journal of Chemical Physics*. As intended, it will also draw papers from a wide range of specialist journals.

The first nine issues of *Langmuir* include many examples of papers which could have been appropriately submitted to one or other of the alternative journals. It is, however, still too early to assess the degree to which it is succeeding in covering its intended field. Most areas are well represented, especially gas-solid systems, surface photochemistry and spectroscopy, insoluble monolayers and electrochemistry. But the coverage of heterogeneous catalysis, as distinct from chemisorption studies, is light, and there are disappointingly few papers on disperse colloids. The quality of the papers is generally high, while the short time to publication, if maintained, will be an attraction to prospective authors.

While one hesitates to approve of yet another new journal, *Langmuir* undoubtedly has an important role to play in the development of surface and colloid chemistry: indeed, it is a pity that the ACS did not enter the field 20-30 years ago! □

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Different package deals

J.W. Emsley

Molecular Crystals and Liquid Crystals: Letters Section. Editor-in-chief M.M. Labes. *Gordon & Breach*. 12/yr. UK £106 (corporate), £84 (institutional), £42.40 (individual); North America \$128 (corporate), \$102 (institutional), \$51.20 (individual).

Molecular Crystals and Liquid Crystals: Bulletin. Editor-in-chief M. M. Labes. *Gordon & Breach*. 12/yr. UK £42 (corporate), £34 (institutional), £16.80 (individual); North America \$50 (corporate), \$40 (institutional), \$20 (individual).

BOTH of these publications are adjuncts of *Molecular Crystals and Liquid Crystals*. The *Letters* used to be published as part of the parent journal, and their issue separately is justified by the editor as a consequence of the increasing number of papers submitted. It is also claimed that there was "a growing interest on the part of subscribers"! To be fair, the editorial does not say which interests of the subscribers were growing, but the implication is that they wanted the *Letters* to be published separately. I can only comment that I find it hard to believe that many subscribers welcome the fragmentation of a journal, particularly one that involves an increase in cost.

The *Letters* aim to provide a means of rapid publication, with an average delay of two months, and to achieve this the contributions are produced directly from the authors' camera-ready typescript. The

result, inevitably, is considerable variation in the quality of the typography. More importantly, such rapid publication can be achieved only by a reduction in the time spent on refereeing and editing. On the evidence of the issues available to me, the standard of papers is high although very few of them justified rapid publication.

The *Bulletin* contains titles and abstracts of papers to be published in the parent journal and hardly justifies a separate publication. In addition, the intention is to publish the titles of papers given at conferences, and to include news items of interest to the subscribers. This raises the question of to whom these journals are addressed. The stated scope embraces liquid crystals, low-dimensional solids and

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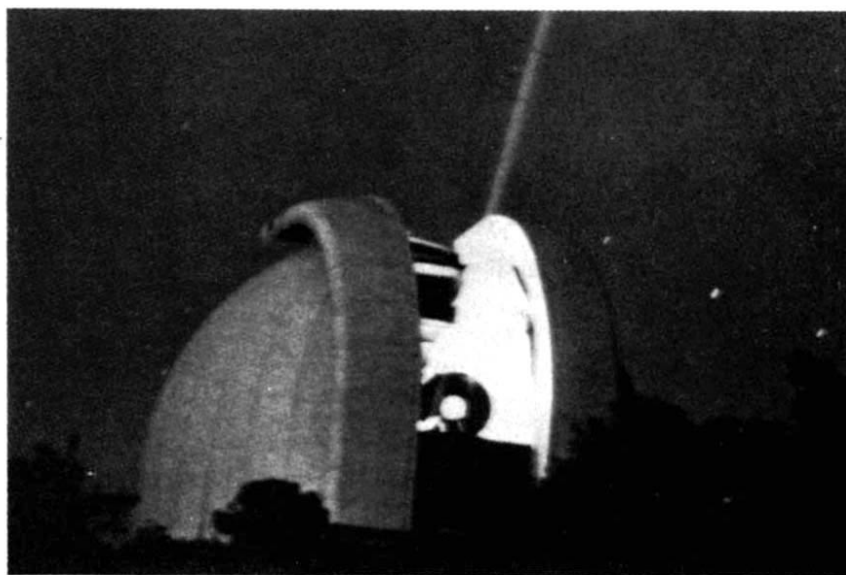
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MOLECULAR CRYSTALS AND LIQUID CRYSTALS

LETTERS SECTION

molecular crystals. In the issues I read the papers on liquid crystals formed a large majority. These materials are the subject of considerable industrial as well as academic attention, and there is undoubtedly a case for a specialized journal dealing with the many different aspects of research and development on them. However, readers interested in liquid crystals will have only a passing interest in either low-dimensional solids or molecular crystals, and certainly those involved in studies of the latter two subjects will find little need for these publications. □

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Shooting the Moon – lunar laser-ranging at the University of Texas McDonald Observatory. The illustration is taken from *Was Einstein Right? Putting General Relativity to the Test*, by Clifford M. Will, to be published next week by Basic Books. The book will be reviewed in *Nature's* Autumn Books supplement on 13 November.

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BECKMAN

Renewable hopes in energy

Brian Norton

Solar and Wind Technology. Editor-in-chief A.A.M. Sayigh. Pergamon. 4/yr. UK £70 (institutional), £31 (individual); North America \$115 (institutional), \$51 (individual); elsewhere DM 330 (institutional), DM 143 (individual).

EVEN before the recent fall in the price of oil, governments in the developed countries had reduced their funding of research into effective ways to harness renewable forms of energy. The time may thus seem inopportune to launch a new journal in this apparently already over-subscribed field. This is not, however, the case. In many developing countries, the reduction in dollar-denominated prices for crude oil have been offset by local currency devaluations and by comparatively high distribution costs for fuel. The consequent high energy-costs in many parts of the Third World, combined with sunny climates, therefore, favour the economic viability of many solar applications, and have stimulated significant research there. The focus of these efforts is on the use of solar and wind energy for power generation, drying, irrigation and refrigeration. The work tends to be applied or developmental and is often specific to a particular geographical area. The diffi-

culty has been to find an appropriate avenue for the publication of the fruits of this research.

Solar and Wind Technology aims to provide this group with a means of rapid, peer-reviewed publication. In this it can be considered a success: in the first four issues, over 64 per cent of the papers originated in developing countries. That this well-produced journal is published for the Arab Section of the International Solar Energy Society with the financial assistance of UNESCO is reflected in the make-up of the editorial board. Together with the absence of page charges, this should help attract papers from the Third World.

Papers are generally accepted within two months of receipt, and subsequently published within six months. This would seem to preclude adequate peer-review: however the quality of the papers, although variable, is generally good. Most issues comprise full papers and technical notes: the criteria for deciding into which category an item should fall is unclear, however, as many of both are five or six pages long.

Solar and Wind Technology fills a need and should do well if those libraries that serve its intended readership in the developing world can afford the foreign exchange to purchase it. I wish it well. □

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Turning numbers on their head

R.E. Burge

Inverse Problems: An International Journal of Inverse Problems, Inverse Methods and Computerized Inversion of Data. Honorary editor P.C. Sabatier. Institute of Physics. 4/yr. UK £130; North America \$220.

IN RECENT years there have been numerous international conferences on topics directly or indirectly concerned with inverse problems. As further evidence of the rise in interest in the subject, this new journal was launched in February 1985.

The inverse problem, or the determination of cause from effect, is universal in physics. It has an obvious expression in the interpretation of an image from which the nature and properties of a scatterer are to be inferred, but the journal's scope is wider than this. The intended audience is pure and applied mathematicians and physicists, but also included are articles on

topics of interest to specialists (for example the investigation of ocean flow by its acoustic properties, or the interpretation of small-angle inelastic electron scattering as related to electron energy-loss spectra).

A criticism of the material published to date is that the problems are divorced from their usual context in, for example, acoustic imaging, tomographic reconstruction or super-resolution. On the other hand, one of the journal's strengths is that it does provide a focus for a range of

Inverse Problems

mathematical approaches and their applications to problems with a common root, thereby providing a place for discussion of analogies and correspondences between different inverse problems and different approaches to their solution. Apart from formal papers, the journal includes brief letters to the editor on technical points. The date of receipt of contributions is noted, and thus far the record for speed of publication is respectable.

From a technical point of view, work involving inverse problems has two

aspects: first, fundamental research related to (for example) optimization theory and the solution of integral and partial differential equations; and, secondly, problems of the interpretation of experimental data (for example from neutron scattering experiments) and of signal restoration, where numerical analysis and the development of computer algorithms is of principal importance. It is valuable to have a publication which brings together material on these two kinds of problem, though it seems unlikely that the audience for each aspect will fully overlap. The range of applications is increasing, how-

ever, with a corresponding rise in the potential number of readers in the primary fields in which such problems crop up. The future of the journal may well depend on the extent to which it strikes a balance between publishing method-related and research-field-related papers. Certainly, however, as far as the present contributors and audience are concerned, the collection together of reports of research involving inverse problems is highly convenient. □

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Fruits of thinking small

Andrew Holmes-Siedle

Superlattices and Microstructures: A Journal Devoted to the Science and Technology of Synthetic Microstructures, Microdevices, Surfaces and Interfaces. Editor-in-chief John D. Dow. *Academic*. 6/yr. UK £80; North America \$152.

THE main business of the microelectronics industry is to make and sell integrated circuits, using mainly silicon and gallium arsenide as working materials and employing the techniques of microfabrication. Recently, these techniques have come to be applied beyond the research and development of circuits, into that of so-called "microstructures".

Thin films play a major role in microfabrication, and one particular method of film deposition — molecular-beam epitaxy — has given rise to an important new range of electronic devices. In this method, the composition of the film, as it is built up, can be modulated regularly so as to give a "superlattice". So special are these two aspects of microfabrication that it was appropriate to launch a journal which embraces them both. It is certain that universities and electronic companies will produce a flood of papers capable of filling its pages to overflowing.

While this is an international journal, it is not surprising that over half of the editorial board is drawn from the United States. However, it is odd that no British institution is represented. This may explain the thin presence of British scientists among the contributors; despite the powerful research going on here in epitaxial growth, there have been no papers from British universities and only a few from industry and government establishments. By contrast, it would appear that Germany and France have some important visiting arrangements with American institutions in this field. These manifest themselves in the form of several excellent

papers from Europeans on superlattice devices.

It is obvious to the solid-state physicist that many new physical phenomena will emerge when we create a synthetic material with a periodic structure not found in nature. When these superlattices are spaced closely enough to form "quantum wells", novel optical and magnetic properties are found and electrons and holes are transported through these layers in a different fashion. Most of the articles in *Superlattices and Microstructures* are devoted to these electronic effects. Exciting though these papers are, however, one would like to see them balanced by reports of work on unconventional microstructur-

SUPERLATTICES AND MICROSTRUCTURES

es such as cantilevers, semiconductors with gridded electrodes, magnetic devices, field emitters, and sensors of pressure and acceleration. Some novel electronic heterojunction and optical devices have been described, and have yielded new acronyms for, for example, the Negative Resistance Field Effect Transistor (NERFET), Coherent Interfaces for Reflection and Penetration (CHIRP) and the Surface Magneto-Optic Kerr Effect. In the contents list of the pertinent issue, however, the latter has lost its capitals, so the title of Moog and Baker's paper appears as "Smoke signals from ferromagnetic monolayers"!

The papers published to date are of a disciplined length and of high quality. At the subscription rate for 1986, the average price per paper is under one pound and represents very good value. If the editors can achieve the balance mentioned earlier, this will be a long-lived and stimulating medium for the reporting of advances in microfabrication. □

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Out of this world

J.C. Leeming

Space Policy. Edited by David Green. *Butterworth*. 4/yr. UK £80; North America \$151; elsewhere £84.

Space Policy is filling an undoubted gap in the literature by providing an opportunity for knowledgeable people to write substantive articles about issues affecting future space developments. The journal's scope is broad and is illustrated by the editor's invitation for submission of "contributions on space . . . developments in their industrial, economic, political, legal and social contexts".

Why should such a journal be of interest to any readers of *Nature*? The answer must lie in the international character of space activity, and the fact that much of it is supported by national governments. The policies of national and of international space agencies provide the infrastructure on which most space and certainly space-science activities depend. Thus, for example, the decision whether Europe should join with the United States, Canada and Japan in building an international space station will influence events into the next century.

It is difficult for those who are not immediately involved to keep abreast of international developments and the underlying policies being pursued by the various agencies. *Space Policy* attempts to provide a source of informed comment on these matters and in so doing to improve upon the gossip that unfortunately characterizes much of the general magazine coverage of space. In this it has made a successful start. The editor has attracted a wide range of articles from an equally wide range of international contributors; thus far, the United States, Europe and China have all been represented. While most of the articles are about civil activities, one issue dealt extensively with the Strategic Defense Initiative and contained not only an article by General Abrahamson, the Director of the SDI Programme, but others giving contrary opinions.

The journal is making a serious effort to reflect a number of views and to make a contribution to the formulation of policies, both national and international. So far it has not attempted to take a particular stance, other than that it is favourably disposed towards space developments. So long as it maintains this perspective, and the current quality of contributions, it will deserve to be read both by those involved in the administration of space policies and those who are affected by the decisions of the space agencies. □

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Computer Science

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Using Computers: Human Factors in Information Systems. By RAYMOND S. NICKERSON. MIT Press:1986. Pp.434. ISBN 0-262-14040-3. \$22.50.

Space Science

The Cosmic Inquirers: Modern Telescopes and Their Makers. By Wallace Tucker and Karen Tucker. *Harvard University Press*:1986. Pp.221. Hbk ISBN 0-674-17435-6. Hbk £16.95.

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Guide to Observing the Moon. By the British Astronomical Association. *British Astronomical Association*:1986. Pp.128. Hbk ISBN 0-89490-085-4. £9.95.

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Mathematics

Current Algebra and Anomalies. By SAM B. TREIMAN, ROMAN JACKIW, BRUNO ZUMINO and EDWARD WITTEN. *Wiley*:1985. Pp.537. Pbk ISBN 9971-966-97-2. £25.85.

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Map Coloring, Polyhedra, and the Four Color Problem. *Dolciani Mathematical Expositions, No.8.* By DAVID BARNETTE. *The Mathematical Society of America*:1986. Pp.168. Hbk ISBN 0-883853094. £27.60.

Markov Processes: Characterization and Convergence. By STEWART N. ETHIER and THOMAS G. KURTZ. *Wiley*:1986. Pp.534. Hbk ISBN 0-471-08186-8. £49.10, \$47.50.

Random Walks and Electric Networks. *The Carus Mathematical Monographs, No.22.* By PETER G. DOYLE and J. LAURIE SNELL. *The Mathematical Association of America*:1984. Pp.159. Hbk ISBN 0-88385-024-9. £22.

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A.M. Turing's ACE Report of 1946 and Other Papers. B.E. CARPENTER and R.W. DORAN (eds). *The Charles Babbage Institute, Reprint Series for the History of Computing. The Massachusetts Institute of Technology*:1986. Pp.140. Hbk ISBN 0-262-03114-0. \$20.

Technology

Advances in Energy Systems and Technology. Volume 5. PETER L. AUER and DAVID DOUGLAS (eds). *Academic Press Inc*:1986. Pp.318. Hbk ISBN 0-12-014905-2. Hbk \$64.00; £62.50.

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